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MYCOLOGIA

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VOL. XLII JANUARY–FEBRUARY, 1950 NO. 1

SEVERAL SPECIES OF DACTYLELLA AND DACTYLARIA THAT CAPTURE FREE-LIVING NEMATODES

CHARLES DRECHSLER[1]

(WITH 15 FIGURES)

Owing to their production of colorless pluriseptate conidia on tall colorless conidiophores, six clampless nematode-capturing hyphomycetes, evidently distinct from all previously described related forms of similar predacious character, are herein set forth as new species of *Dactylella* and *Dactylaria*. It is believed that the several species are distributed between the two genera in tolerable conformity with established usage, though as has been pointed out earlier (**13**: 467), the distinction between a solitary and a capitate sporulating habit is sometimes rather difficult to apply among members of the predacious series. The several new species came to light in agar plate cultures which after being overgrown with *Pythium* mycelium had been further planted with small quantities of decaying vegetable detritus from different localities; the decaying material in each instance supplying not only the nematodes that through rapid multiplication soon infested the agar abundantly but also the fungi by which the animals were subsequently destroyed in large numbers. Occasion is taken, besides, to set

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forth in a provisional way the predacious organs of a related nematode-capturing hyphomycete that so far has not been seen giving rise to reproductive apparatus. Some attention, furthermore, is given to a mucedinous form held referable to *Gonatobotrys simplex* Corda (**9**), since in its mycelial habit it offers suggestive resemblances to one of the six new species, and in its sporulating habit shows general parallelism more especially with the repeatedly nodose species of *Arthrobotrys* so widely active in the capture of eelworms.

A DACTYLELLA WITH SMALL CONSTRICTING RINGS AND MOSTLY BISEPTATE OR TRISEPTATE CONIDIA

Several Petri plates of maizemeal agar which after being overgrown with mycelium of *Pythium ultimum* Trow had been further planted with small quantities of leaf mold taken from the floor of pine (*Pinus* spp.) woods in Yellowstone National Park on August 18, 1947, became abundantly infested with eelworms during the ensuing weeks. By far the larger number of individual eelworms were conspicuously slender in shape and belonged, according to Dr. G. Steiner, to two species of *Plectus*. On microscopic examination 42 days after the leaf mold had been added the eelworms, especially those of the slender species, were found being killed over a wide area by a strangling hyphomycete differing in appearance from any known form of similar biological habit.

The vegetative mycelium of the hyphomycete came forth here and there from the deposits of opaque detritus to extend sparsely into the transparent agar. It was composed of sparingly branched colorless filaments partitioned at moderate intervals by cross-walls. At somewhat longer intervals the hyphae bore sturdy three-celled rings (FIG. 1, *A;* FIG. 2, *A, a, b*) which like the similar organs of other nematode-strangling forms were usually oriented in a plane perpendicular to the hyphal axis, and consequently were most often seen in edgewise view. The cellular make-up of the rings was better revealed in the occasional specimens that had been pushed sideways through the jostling of vigorous nematodes to be brought conveniently into lateral view (FIG. 1, *B–E; F, a, b.* FIG. 2, *B–E*); though such change in position entails some distortion of the stalk and at times also of the adjacent portion of mycelial filament. As

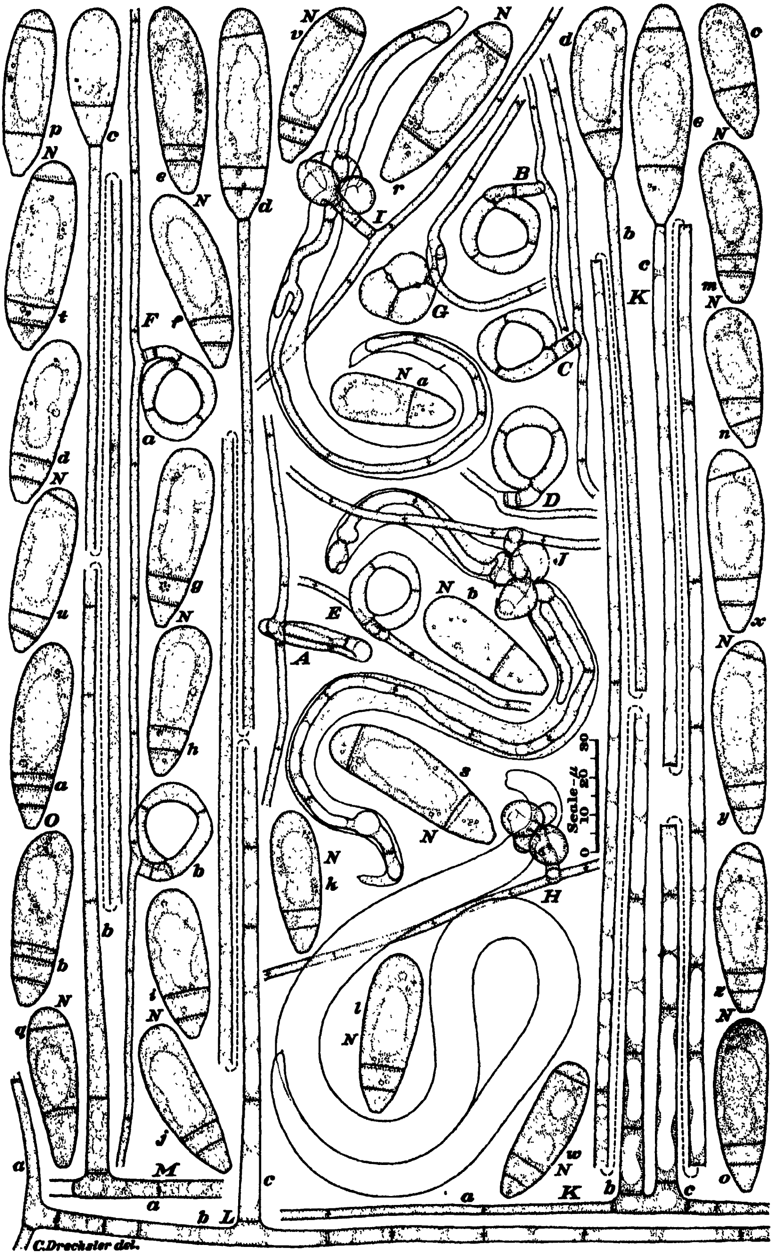

FIG. 1. *Dactylella stenobrocha.*

in other species the stalks were regularly composed of two cells. In relatively short stalks the proximal cell was often shorter than the distal cell (FIG. 1, *C; D; F, a, b*), thereby providing similarity to the strangling species I have described earlier under the binomials *Arthrobotrys dactyloides* (**12**: 482–487), *Dactylella bembicodes* (**12**: 487–492) and *Dactylaria brochopaga* (**12**: 514–518). Yet frequently, again, the two cells were of about equal length (FIG. 1, *B;* FIG. 2, *B–E*) somewhat as in my *Dactylella coelobrocha* (**19**). The rings themselves closely resembled the corresponding organs of *Arthrobotrys dactyloides, Dactylella bembicodes* and *Dactylaria brochopaga* in always showing smooth curvature along their rounded triangular inner profile as well as along their more nearly circular outer profile. Their three arcuate cells were never found showing at their inner profile the curious median bulge whereby the aperture of the constricting ring in my *Dactylella doedycoides* (**13**) and my *Dactylella heterospora* (**15**) is given a scalloped outline suggestive of trefoil ornamentation. They tapered moderately toward the cross-walls separating them, like the arcuate cells of most nematode-strangling species, and thus did not share the more pronounced taper characteristic of the arcuate cells in *Dactylella coelobrocha.* In general the rings here appeared of smaller and less variable size than those of any nematode-strangling form hitherto described with the exception possibly of my *Trichothecium polybrochum* (**12**: 535–538). The range of dimensions shown by them would in most allied species include only the constricting rings of small and medium sizes.

Here and there in the cultures where the fungus made its appearance rings were found that had closed emptily (FIG. 1, *G;* FIG. 2, *F*), thereby revealing advantageously the change of the three component cells from an elongated arcuate to an obese orbicular shape. Usually, however, the rings became closed in capturing individual specimens of the two slender species of *Plectus* (FIG. 1, *H–J.* FIG. 2, *G; H, a; I; J*) abundantly infesting the agar substratum. In some instances a ring that had closed emptily (FIG. 2, *H, b*) was found near one that held a captured nematode, suggesting that disturbance by the struggling captive, or perhaps impact of the animal's body against the flat side of the ring, may have supplied the stimulus that resulted in unprofitable closure. Captured eelworms were always found indented about equally by all three swollen cells; the ring here manifestly operating in the manner most widely prevalent among nematode-strangling forms rather than in the manner distinctive of *Dactylella coelobrocha,* which commonly grips the animal between the first and second cells. As long as a captured eelworm continued struggling with some vigor, no change was observable near the constricted ring. Once the animal was capable only of feeble movements its integument was penetrated by one or more of the three swollen cells, and into its fleshy body globose protuberances were intruded forward and backward (FIG. 1, *H*). These protrusions soon grew out distally into assimilative hyphae which finally extended the invasion through virtually the whole length of the captive (FIG. 1, *I*). Frequently in very slender eelworms only a single assimilative hypha was extended forward and backward from the enveloping ring (FIG. 1, *I*), but in somewhat stouter animals the median portions usually came to be occupied by two hyphae (FIG. 1, *J*. FIG. 2, *H*, *a*; *J*). At the anterior end of the animal, as also at its tail end, the assimilative hyphae often showed marked distension in one or two terminal segments (FIG. 1, *I*, *J*; FIG. 2, *H*, *I*), a type of modification noted previously in *Arthrobotrys dactyloides* and *Dactylella coelobrocha.* During the progress of invasion and for some time afterwards the assimilative hyphae were obscured badly, owing to the globuliferous character of the animal's degenerating contents, but as these contents gradually diminished the assimilative hyphae emerged into view more and more clearly. When the animal's substance was in large part depleted the assimilative hyphae became noticeably vacuolate (FIG. 1, *J*; FIG. 2, *H*, *J*). The vacuoles increased steadily in volume on further reduction of the materials in the eelworm, until finally the hyphal envelopes were no less empty of living protoplasm than the animal's integument surrounding them.

With ample nourishment thus being obtained through expropriation of captured eelworms, the fungus produced conidiophores at variable intervals along the mycelial filaments (FIG. 1, *K*, *a*; *L*, *a*) on the surface of the agar substratum. The conidiophores (FIG. 1, *K*, *b*, *c*. FIG. 2, *K*, *a*; *L*, *a*) here consisted of sturdy erect septate hyphae, about 0.5 mm. high, that tapered very gradually toward the tip where each bore a solitary conidium (FIG. 1, *K*, *d*, *e*. FIG. 2, *K, b; L, b*). After the conidium had been formed the conidiophore, while still in an erect posture, sometimes lost much (FIG. 3, *A*) or all (FIG. 3, *B*) of its protoplasmic contents. Sometimes, again, it would fall over on the substratum while all (FIG. 1, *L, b*) or some (FIG. 1, *M, a*) of its segments remained alive, and then in many instances would give rise, usually from one of its proximal segments, to a secondary conidiophore (FIG. 1, *L, c; M, b*) which, like its parent, bore a single conidium (FIG. 1, *L, d; M, c*).

The conidia produced by the fungus in nematode-infested agar cultures were regularly of elongated ellipsoidal shape. Many though not all of them were slightly wider in the distal portion than in the proximal portion. Usually the distal end was broadly rounded, whereas the lower half of the spore commonly tapered toward the somewhat rounded truncate base. They were divided variously by cross-walls ranging in number from one to three. In the uniseptate conidia (FIG. 1, *N, a–c;* FIG. 2, *M, a*), which were relatively infrequent, the septum nearly always delimited a long distal cell from a shorter proximal cell. The biseptate conidia, which were abundant, consisted of two short cells and one long cell; the long cell in some instances (FIG. 1, *N, d–o;* FIG. 2, *M, b–m*) surmounting the two short ones, and in other instances (FIG. 1, *N, p–s;* FIG. 2, *M, n, o*) being placed between them. The almost equally numerous triseptate conidia contained three short cells and one long cell; the long cell here most often occurring in penultimate position (FIG. 1, *N, t–z.* FIG. 2, *M, p–z; N*) between a short apical cell and two short proximal (*i.e.*, basal and parabasal) cells, though sometimes it occupied a terminal position (FIG. 1, *O, a, b*). In mature conidia, as a rule, the large cell contained a rather large elongated vacuole around which subspherical granules were often found scattered a little more thickly than elsewhere in the protoplasm. Conidia after falling on a moist substratum were often found sending up one (FIG. 2, *O, P*) or two (FIG. 2, *Q*) tapering aerial hyphae from any of the small cells. These aerial hyphae were noticeably more slender than ordinary germ hyphae extended by the conidia (FIG. 2, *R*) when immersed in fresh water or in some agar medium.

The fungus was isolated by removing conidia aseptically from the tall conidiophores to Petri plates of sterile maizemeal agar, the

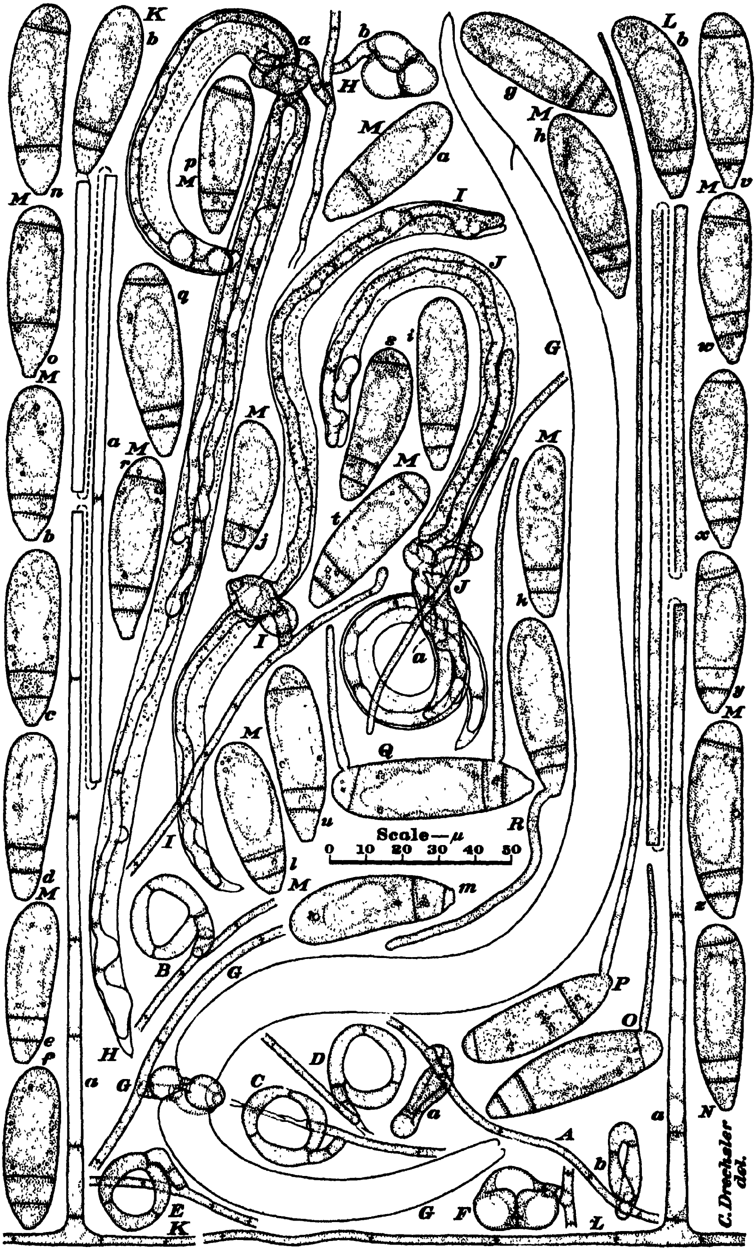

FIG. 2. *Dactylella stenobrocha.*

transfer being accomplished readily by touching the conidia with a small slab of sterile agar held on a flamed platinum spatula. In pure culture on maizemeal agar kept at a temperature of 20° C. it grows with moderate rapidity, usually without forming any predacious rings. Predacious rings corresponding accurately both in their cellular make-up and in their relatively small dimensions to those produced in nematode-infested materials have, however, been found present abundantly in pure cultures of the fungus in tubes of maizemeal agar that had been stored for over eleven months at a temperature of 5° C. in containers affording some protection against evaporation. As the aging cultures, on microscopical examination, showed no contamination by any bacteria, nor any admixture of other fungi, nor any infestation by animals, it would seem that the new hyphomycete can give rise to predacious rings, even though perhaps only rather slowly, without any chemical or physical stimulus from alien organisms.

Although the fungus sporulates rather freely in pure culture on maizemeal agar at temperatures near 20° C., the conidial apparatus produced here differs in dimensions from that formed on nematode-infested substratum. The conidiophores (FIG. 3, *C–E*) are usually much shorter, their height ranging ordinarily from 150 to 250 μ. The conidia likewise are shorter, commonly varying from 30 to 40 μ in length; and as their width is reduced only little, if at all, they are generally of smaller size and plumper shape. Uniseptate conidia (FIG. 3, *F, a–o*) are produced more frequently in pure culture, though showing usually the same manner of partitioning into a longer distal cell and a shorter proximal cell that is most prevalent in nematode-infested cultures. Biseptate conidia here, much as in nematode-infested cultures, may have both cross-walls placed toward the basal end (FIG. 3, *G, a–h*) or may show a more symmetrical arrangement in having one cross-wall near the basal end and the other near the distal end (FIG. 3, *G, i–p*). Triseptate conidia (FIG. 3, *H*), if formed less frequently than in nematode-infested cultures, usually display similar disposition of cross-walls. The fungus evidently sporulates also at relatively low temperatures, since in pure culture in tubes of maizemeal agar stored at 5° C., it showed on examination after eleven and one half months an abundance of new living conidia. These conidia seemed

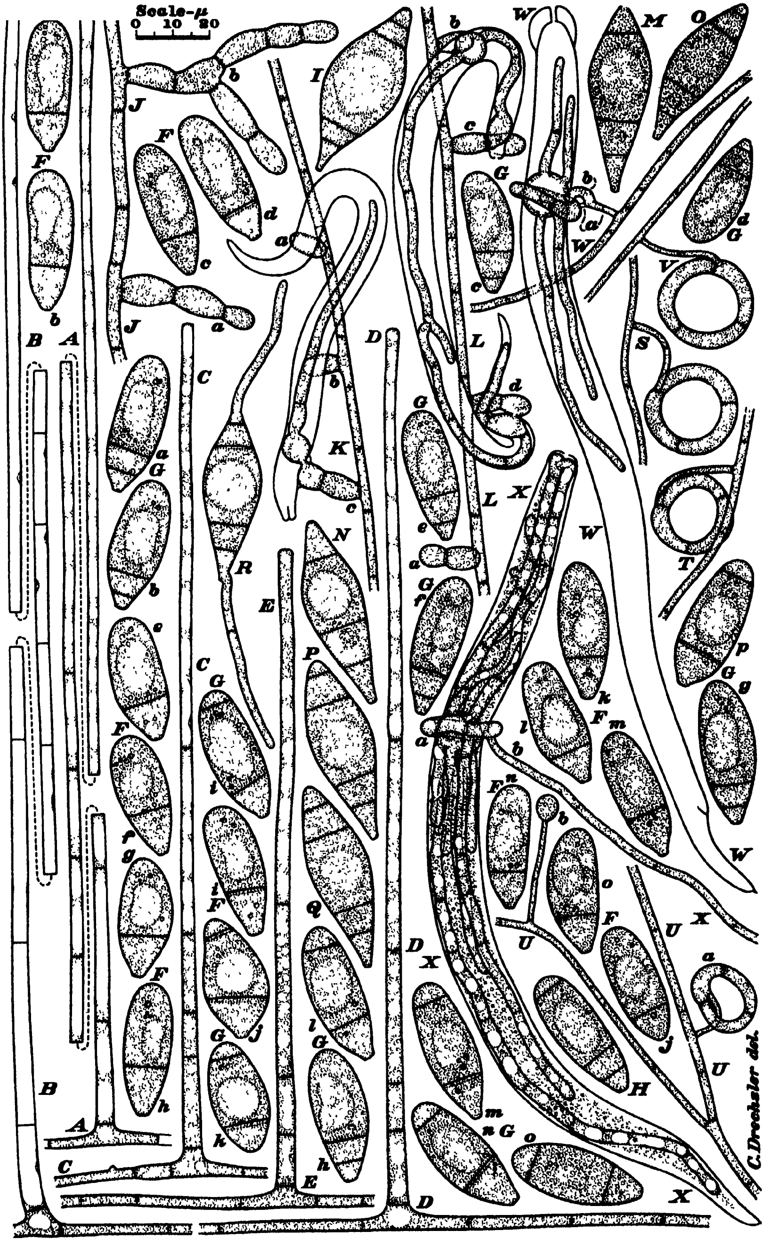
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FIG. 3. *A–H, Dactylella stenobrocha; I, D. aphrobrocha; J–R, D. cionopaga; S–X*, Unnamed Hyphomycete.

generally larger than those produced at 20° C., and were given more frequently to rather pronounced curvature. The conidiophores had a tendency toward production of successive spores following repeated elongation.

Despite the promiscuous variations of its conidia in pure culture, the fungus was never found producing spores that could be regarded as belonging in a different category from the elongated ellipsoidal conidia produced in nematode-infested cultures. It was never observed giving rise to any supplementary reproductive bodies comparable, for example, to the uniseptate allantoid conidia of *Dactylella heterospora*, or to the elongated globuliferous spores of *Dactylella doedycoides*. It has never been seen provided with chlamydospores such as are present abundantly in cultures of *Arthrobotrys oligospora* Fres., nor with distinctive aggregations of enlarged indurated mycelial cells of the sort familiar in cultures of *Dactylella heterospora*, though after several months some of its wider hyphae often appear slightly indurated in that their walls are then thickened and their contents have become partly globulose. While its conidia, in their elongated shape, most nearly resemble the large regularly biseptate conidia of *Dactylella heterospora*, they have never been found produced in submerged positions under the surface of agar substrata, their contour at the base is rounded truncate rather than sharply truncate, and they frequently contain three cross-walls—so frequently, indeed, that the triseptate condition may well be regarded as being typical of the species. In their outward shape they differ markedly from the obese conidia of *Dactylella doedycoides*, as well as from the swollen turbinate conidia of *Dactylella bembicodes*; while their generally greater dimensions and different arrangement of septa distinguish them from the conidia of *Dactylaria brochopaga*, which are usually borne in capitate clusters rather than singly.

A term having reference to its relatively small constricting rings is deemed suitable as a specific epithet for the fungus.

Dactylella stenobrocha sp. nov.

Mycelium sparsum; hyphis hyalinis, mediocriter septatis, plerumque 1.7–3.7 μ crassis, hic illic ex ramulis bilocularibus (rarius trilocularibus) vulgo 8–14 μ longis et 3–4.5 μ crassis laqueos circulares 20–31 μ (vulgo circa 23 μ) latos proferentibus qui in 3 cellulis arcuatis 14–25 μ (vulgo circa 17 μ) longis medio 4.4–5.5 μ extremo circa 3 μ crassis consistunt et foramen rotundum vel rotundo-triangulum 11–21.5 μ (vulgo circa 13 μ) latum circumdant; vermiculo nematoideo in laqueum apertum errato omnibus tribus cellulis abrupte se contrahentibus, itaque animal captivum magnopere comprimentibus, mox id trucidentibus, integumentum ejus perforantibus, hyphas intus evolventibus quae carnem exhauriunt; hyphis assumentibus incoloratis, septatis, magnam partem 2–3.5 μ crassis, sed saepe in cellulas 4–8 μ crassas abeuntibus. Hyphae fertiles incoloratae, erectae, vulgo 425–550 μ altae, basi 4.5–6.5 μ crassae, sursum leniter usque 2.5–3 μ attenuatae, 3–10 septatae, apice quandoque leviter latescentes, ibi saepe 3–4.5 μ crassae, unum conidium ferentes; conidiis hyalinis, elongato-ellipsoideis vel late digitiformibus vel interdum aliquantulum clavatis, rectis vel leniter curvatis, basi aliquid rotunde truncatis, apice late rotundatis, vulgo 34–56.5 μ longis, 12.5–16.5 μ crassis, 1–3 septatis, in una grandiore cellula et 1–3 minoribus cellulis consistentibus, grandiore cellula (in uniseptatis sporis plerumque cellula superiore, in pluriseptatis sporis plerumque cellula ultima aut paenultima) vulgo 21–38 μ longa, minoribus cellulis vulgo 3–15 μ longis.

Vermiculos nematoideos diversos praecipue nematoidea gracilia capiens consumensque habitat in humo silvestri in Yellowstone National Park, Wyoming.

Mycelium scanty; vegetative hyphae colorless, septate at moderate intervals, mostly 1.7 to 3.7 μ wide, often especially in presence of nematodes producing mostly underneath and in perpendicular positions approximately circular rings measuring 20 to 31 μ (usually about 23 μ) in outside diameter and composed individually of three arcuate cells 14 to 25 μ (usually about 17 μ) long, 4.4 to 5.5 μ wide in the middle and about 3 μ wide at the ends—the first and third of the cells being united to each other as well as to the distal end of a frequently somewhat curved bicellular (occasionally tricellular) supporting stalk commonly 8 to 14 μ long and 3 to 4.5 μ wide, and all three of the cells having a smooth profile without median protuberance on the side bordering the ring aperture; this aperture being of circular or rounded triangular shape and varying commonly from 11 to 21.5 μ in diameter, most often being about 13 μ wide. After entrance of a nematode into the ring aperture all three arcuate cells abruptly contracting, thereby constricting the animal to death or disabling it, then perforating the integument and extending lengthwise through the body assimilative hyphae for the most part 2 to 3.5 μ wide but often terminating in cells 4 to 8 μ wide. Conidiophores colorless, erect, commonly 425 to 550 μ high, 4.5 to 6.5 μ wide at the base, tapering upward very gradually to a distal width of 2.5 to 3 μ, ultimately becoming divided by 3 to 10 cross-walls, at the apex sometimes widening slightly to a diameter of 3 to 4.5 μ, and bearing there a single conidium. Conidia colorless, elongate elliptical or broadly finger-shaped or sometimes slightly clavate, straight or slightly curved, somewhat rounded truncate at the narrowed base, broadly rounded at the tip, commonly

34 to 56.5 μ long and 12.5 to 16.5 μ wide, divided by 1 to 3 cross-walls into 2 to 4 cells whereof one exceeds the other or others in size, the larger cell (in uniseptate spores usually the distal cell, in pluriseptate spores usually either the apical or the penultimate cell) commonly 21 to 38 μ long, the smaller cells commonly 3 to 15 μ long.

Capturing and consuming different nematodes, especially nematodes of slender body shape, it occurs in leaf mold in Yellowstone National Park, Wyoming.

A DACTYLELLA PRODUCING LONG-STALKED CONSTRICTING RINGS AND QUADRISEPTATE CONIDIA

Six years ago a nematode-strangling fungus resembling *Dactylella bembicodes* in the general appearance of its tall conidiophores and solitary conidia was found developing meagerly in a maizemeal-agar plate culture which after being overgrown with mycelium of my *Pythium salpingophorum* had been further planted with a small quantity of leaf mold gathered in a deciduous wood near Fairfax, Virginia, on November 10, 1942. The fungus arrested attention because its conidia, though of broadly fusiform shape much like those of *D. bembicodes*, were nearly always divided by four rather than by three cross-walls (FIG. 3, *I*). As *Dactylella coelobrocha* had not then been discovered the quadriseptate condition was quite alien to the conidia of any nematode-strangling hyphomycete known at the time. It was noted, moreover, that the stalks bearing the constricting rings were in general a little longer than in *D. bembicodes*. To determine whether the observed differences came about as variations of *D. bembicodes* or derived from a separate species, attempts were made to isolate the fungus by removing its conidia to a sterile agar medium. These attempts failed owing to the unfortunate circumstance that the rather small number of conidiophores available were closely intermingled with taller sporangiophores of a species of *Mucor*. Every lot of conidia removed was in some measure mixed with small spores of the *Mucor*. In all cultures planted with the mixture, the rapidly growing *Mucor* quickly overwhelmed the hyphomycete.

Early in 1948 the same fungus developed abundantly in more than a dozen maizemeal-agar plate cultures which, after being permeated with mycelium of *Pythium debaryanum* Hesse, had been further planted with small quantities of leaf mold kindly gathered by Dr. E. B. Toole in woods consisting mainly of pine (*Pinus*) and oak (*Quercus*) trees near Greensboro, North Carolina, on December 29, 1947. From the lofty conidiophores sent up in scattered positions over extensive tracts of substratum in these cultures, conidia of the fungus were readily transferred to sterile agar without admixture of any alien organism. Ample opportunity was thus provided for comparing the fungus, in pure as well as in nematode-infested cultures, with all known hyphomycetes of similar predacious habit, apart from *Trichothecium polybrochum*, which has not been seen again since it was first encountered in 1933. The fungus from North Carolina was soon revealed as differing decisively from *Dactylella bembicodes* and *Dactylella coelobrocha*. Through its disclosure as a separate species the number of distinct mucedinous forms known to utilize constricting rings in their capture of eelworms is increased to nine.

When sizable portions are cut from pure cultures of the new *Dactylella* and placed on nematode-infested agar in Petri dishes, mycelial filaments grow out somewhat sparingly in all directions from the transferred material into the surrounding medium. While the mycelium developed by the fungus in pure culture on maizemeal agar is regularly free of predacious organs, the hyphae extended into nematode-infested agar promptly give rise to constricting rings at somewhat variable intervals. These rings, much as in allied species, are commonly formed in planes perpendicular or nearly perpendicular to the mycelial filament bearing them (FIG. 4, *A*; *B*, *a*. FIG. 5, *A*). Some of them, on being jostled vigorously by robust eelworms, are turned sideways in noticeable measure (FIG. 4, *B*, *b*; *C*, *a*, *b*; *D*; *E*. FIG. 5, *B*), and a few are turned flatwise (FIG. 4, *F–H*; *I*, *a*, *b*. FIG. 5, *C–H*) so that their cellular make-up is conveniently exposed to view. On several occasions it was possible to observe progressive steps in the development of predacious rings oriented flatwise in slabs of nematode-infested agar mounted on a microscope slide under a cover glass. The developing organ in each instance first became recognizable as a circinately curved lateral branch delimited by a basal septum from the frequently somewhat narrower parent hypha (FIG. 5, *I*, *a*), and filled with

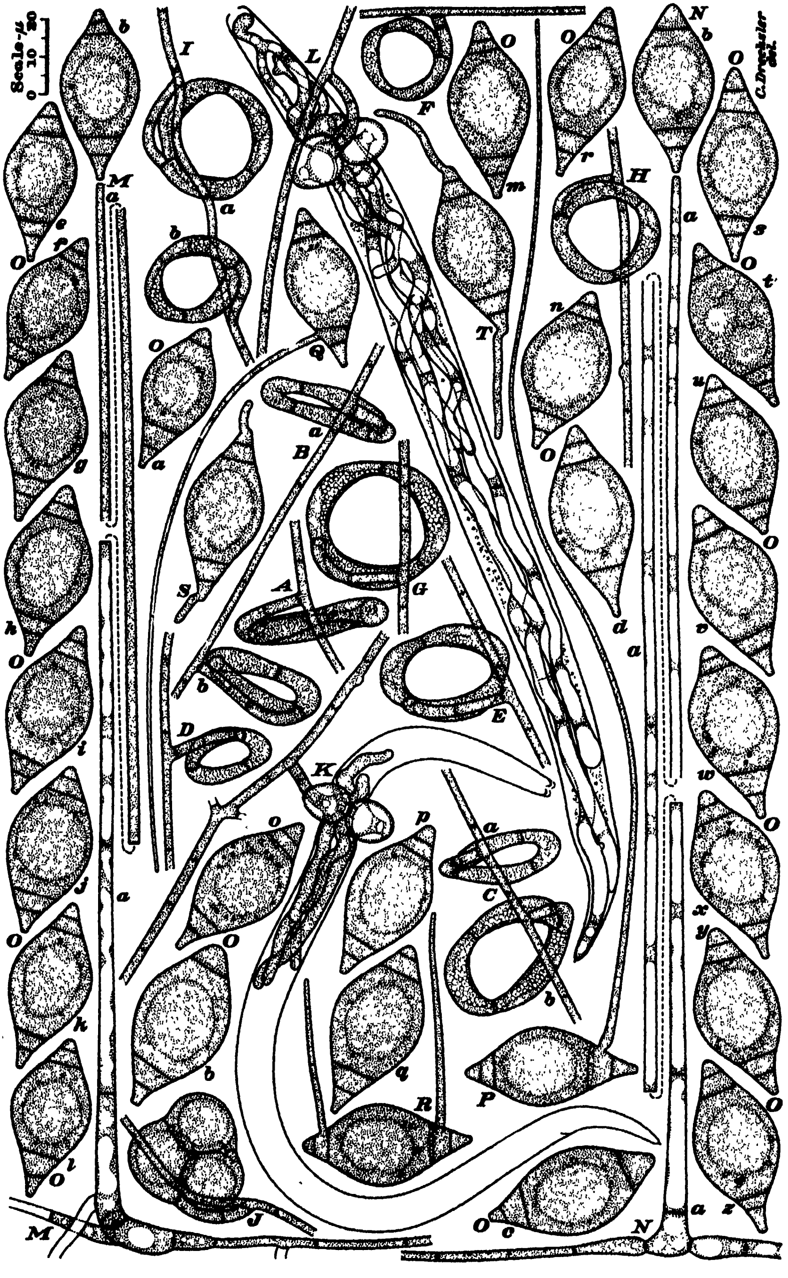

FIG. 4. *Dactylella aphrobrocha.*

protoplasm of nearly homogeneous consistency. Two cross-walls soon appeared in the proximal portion of the branch to delimit the two segments destined to make up the stalk. As the tip of the recurving branch approached the farther septum a spur grew out from the distal end of the second stalk cell to meet it (FIG. 5, *I, b*). The spur often attained a length of 3 or 4 μ before its tip encountered the tip of the branch (FIG. 5, *I, c*). At about the same time another cross-wall was formed to delimit the proximal arcuate segment of the ring that came into being as the juxtaposed tips fused broadly (FIG. 5, *I, d*). Later a second connection was established through hyphal fusion between the basal end of the proximal arcuate segment and the portion of the ring that had originated as a lateral spur The continuity of both anastomosing connections eventually was modified through the formation of a cross-wall in each; one of the cross-walls being laid down in a position corresponding to the base of the lateral spur, whereas the other was laid down midway in the second connection. With the deposition meanwhile of another cross-wall midway between the distal end of the first arcuate segment and the septum formed at what had been the base of the lateral spur, the second and third arcuate segments were delimited from one another, thereby completing the cell divisions required in putting forth a constricting ring of usual structure (FIG. 5, *I, e*).

Departure from the usual cellular structure is observable now and then in instances where the stalk consists of three (FIG. 4, *B, b;* FIG. 5, *J*) rather than two cells. These three-celled stalks commonly exceed in length the arcuate segments of the rings supported by them, whereas the bicellular stalks are most often a little shorter than the associated arcuate segments. However, even the bicellular stalks must be considered relatively long in comparison with those of most allied species, being about equal in length to the rangy stalks of *Dactylella doedycoides* and *Dactylella heterospora.* The arcuate cells in the present fungus lack the median bulge whereby the aperture of the rings in *Dactylella doedycoides* and *Dactylella heterospora* is given a scalloped outline suggestive of trefoil ornamentation. They taper toward their delimiting septa in about the same measure as the homologous cells of all other known nematode-strangling forms apart from *Dactylella coelobrocha.* Apparently the arcuate cells acquire their definitive shape together with their contractile power during their later stages of development. During these later stages (FIG. 5, *I, d, e*) they increase perceptibly in length as the ring enlarges, while their thickness near the septa increases in about the same proportion. In their median region, however, they undergo more pronounced widening, so that their width here often comes to be twice that of the stalk cells with which they had formed earlier a branch of fairly uniform diameter. Distinctive changes take place also in their internal structure. Arcuate cells capable of contraction show along the inner side bordering the ring aperture a rather thin layer of protoplasmic material having a characteristic dense appearance. Material of similarly dense consistency is likewise present in a layer of more variable thickness along the outer side. Between the two dense layers is an irregular elongated region filled with rather faintly visible globules which collectively offer a somewhat foamy appearance when the ring is viewed flatwise. When the ring is viewed edgewise in its normal perpendicular posture the arcuate cells appear as if they were marked by a series of curved transverse striations (FIG. 4, *A*). Presumably the globuliferous core here corresponds to the elongated vacuole or vacuole-like part displayed by the arcuate cells of *Dactylella coelobrocha.*

In nematode-infested cultures predacious organs may be found here and there that have closed emptily (FIG. 4, *J*), thus showing conveniently the pronounced contraction undergone by the three component cells in changing from an arcuate to an orbicular shape. Ordinarily, of course, closure of the organ results in the capture of a nematode; the animal being gripped most often at its anterior end (FIG. 4, *K; L;* FIG. 5, *K–M*), though occasionally it is held by the tail end (FIG. 5, *N*). All three swollen cells of the closed ring are indented broadly and deeply into the yielding body of the eelworm. After the captive's struggles have become feebler, presumably from exhaustion, its integument is narrowly perforated in two or three places by slender protuberances from the swollen cells. Each protuberance, on reaching the fleshy interior, widens markedly (FIG. 5, *K*) to become recognizable as an assimilative hypha (FIG. 4, *K*). The several assimilative hyphae elongate until the animal's body is invaded from head to tail (FIG. 4, *L;* FIG. 5, *L, N*). During their period of active growth the assimilative hyphae show only rather scanty septation, but since they continue to form cross-walls for some time after growth ceases, they eventually are divided into segments mostly 10 to 50 μ long. In some instances the main assimilative filaments bear a moderate number of branches and spurs (FIG. 5, *M*), but in other instances, again, branching is infrequent (FIG. 4, *L*; FIG. 5, *N*), or even wholly absent (FIG. 5, *L*). Hyphal fusions between assimilative hyphal elements are to be found occasionally (FIG. 5, *M*). Although now and then an assimilative hypha terminates in a noticeably expanded cell (FIG. 4, *L*), this sort of modification would seem hardly frequent enough to be regarded as a characteristic feature of the species.

The assimilative hyphae in their progressive invasion of a captured eelworm bring about globulose degeneration of its fleshy substance, and thereby are badly obscured from view, especially if the animal's body is relatively stout. After the globulose materials have been largely absorbed by them, the hyphae become more clearly visible. On further depletion of the animal's substance, vacuoles make their appearance in the hyphal segments (FIG. 4, *L*; FIG. 5, *N*). With continued enlargement of these vacuoles, the protoplasmic contents of the assimilative hyphae are steadily withdrawn backward into the mycelial filament bearing the predacious organ, until ultimately the hyphal envelopes, like the collapsing integument surrounding them, are left entirely empty. The transfer of hyphal contents backward into the mycelial filament is sometimes accomplished by way of the stalk on which the constricting ring was produced (FIG. 4, *L*). Often, however, the original stalk degenerates (FIG. 5, *M*, *a*; *N*, *a*), very probably as the result of injury sustained from the violent struggles of the captured eelworm. Where such degeneration occurs a new hyphal element is produced to provide a passageway for the backward movement of fungus protoplasm. Usually the new hyphal element (FIG. 5, *M*, *b*) connects the mycelial filament with one or another of the three swollen cells. Somewhat rarely, where the swollen cells have all suffered some injury, the new connection may be established directly between the mycelial filament and the proximal hyphal cell within the animal (FIG. 5, *N*, *b*). In its ready development of new hyphal connections the fungus shows obvious similarity to *Dactylella doedycoides* and *Dactylella heterospora,* which, as has been noted, likewise bear

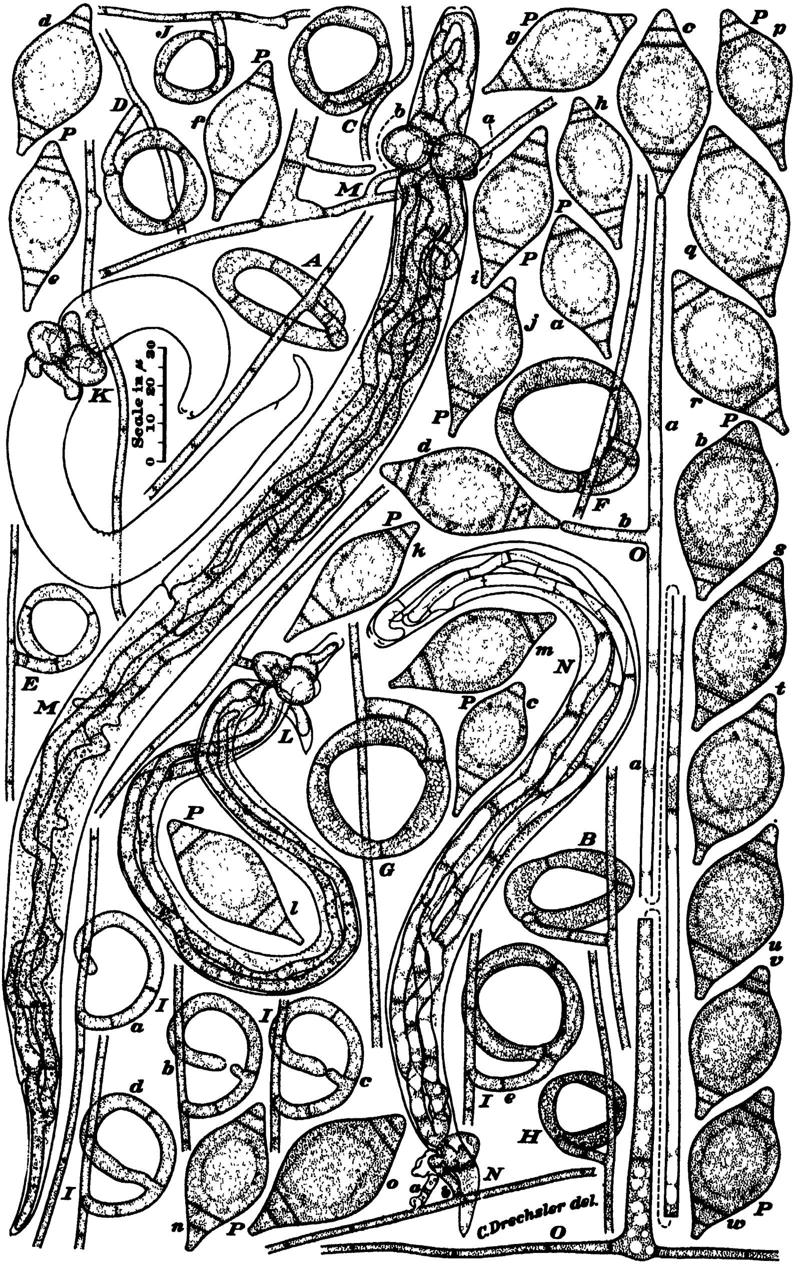

FIG. 5. *Dactylella aphrobrocha.*

their predacious rings on relatively long stalks easily injured by violent struggles of captured eelworms.

The scattered erect conidiophores sent up by the fungus from hyphae prostrate on nematode-infested substrata resemble those of *Dactylella bembicodes* in their stature and gradual upward tapering (FIG. 4, *M, a; N, a*). As a rule they give rise terminally to a single conidium (FIG. 4, *M, b; N, b*), but now and then a conidiophore (FIG. 5, *O, a*) will put forth a lateral branch (FIG. 5, *O, b*) some distance below the tip, and thus is enabled to form two conidia (FIG. 5, *O, c, d*). Although on the whole the conidia here are somewhat longer and wider than those of *Dactylella bembicodes,* and in general appear to have their greatest width rather more accurately midway between base and apex, the differences in size and shape they present in comparison with the widely distributed constricting species made known earlier are not very pronounced. In some instances they contain three cross-walls, which often delimit a small apical cell, a large ventricose penultimate cell, a small parabasal cell, and a small basal cell (FIG. 4, *O, a–c;* FIG. 5, *P, a–c*), thus bringing about the usual cellular arrangement found in spores of *Dactylella bembicodes.* Often again, however, in triseptate conidia it is the parabasal cell that is large and ventricose, while the penultimate cell, as also the apical and basal cells, will be relatively small (FIG. 4, *O, d*). Although triseptate conidia are formed rather abundantly in pure culture on maizemeal agar, by far the greater number of conidia produced by the fungus in nematode-infested cultures are symmetrically quadriseptate (FIG. 4, *O, e–z;* FIG. 5, *P, d–w*); a massive ventricose cell with a large vacuole being found in median position between two smaller proximal (basal and parabasal) cells below and two smaller distal (penultimate and apical) cells above. As conidia with more than four cross-walls are virtually unknown here, the quadriseptate condition may safely be regarded as being definitive in mature spores, whereas the positions of the cross-walls in many triseptate specimens taken from actively sporulating nematode-infested cultures give ample reason for suspecting that had no disturbance intervened a fourth transverse partition would have been formed. Accordingly the data on conidial dimensions given in the diagnosis were derived, much as in the case of *Dactylella coelobrocha,* mainly from quadriseptate specimens taken at random in mounts prepared from abundantly sporulating nematode-infested agar plate cultures. One hundred measurements for length, expressed in the nearest integral number of microns, showed a distribution as follows: 41 μ, 1; 42 μ, 2; 43 μ, 1; 44 μ, 15; 45 μ, 13; 46 μ, 21; 47 μ, 17; 48 μ, 11; 49 μ, 6; 50 μ, 3; 51 μ, 6; 52 μ, 2; 54 μ, 1; 55 μ, 1. The measurements for greatest width, relating to the same 100 conidia, were distributed thus: 17 μ, 1; 18 μ, 1; 19 μ, 7; 20 μ, 7; 21 μ, 21; 22 μ, 22; 23 μ, 25; 24 μ, 12; 25 μ, 3; 26 μ, 1; while the median cells of the 100 specimens gave values for length as follows: 23 μ, 2; 24 μ, 1; 25 μ, 7; 26 μ, 20; 27 μ, 25; 28 μ, 23; 29 μ, 14; 30 μ, 6; 31 μ, 1; 34 μ, 1. Although the conidia here are substantially shorter than those of *Dactylella coelobrocha*, the two species show rather little difference with respect to the lengths of the parabasal, median, and penultimate cells. Manifestly the conidia of *Dactylella coelobrocha* owe their greater length, as also their markedly different appearance, to their characteristically protracted basal and apical cells, which are conspicuously longer than the concomitant parabasal and penultimate cells, respectively. Often the conidia of the present fungus, while still borne aloft on the conidiophores, will put forth a slender aerial hypha, usually from the small parabasal cell (FIG. 4, *P*, *Q*), but sometimes also from the small penultimate cell (FIG. 4, *R*). In general these aerial hyphae resemble the slender outgrowths extended by the conidia of *Dactylella coelobrocha*, but are perhaps somewhat less rigid. As in related species they seem to be produced more profusely under warm than under cool conditions. When mature conidia fall on a moist substratum or are shallowly immersed in water, they germinate in commonplace manner by putting forth a germ hypha from the small apical cell and from the small basal cell (FIG. 4, *S*, *T*).

In recalling the globuliferous core of its contractile arcuate cells a term compounded of two words meaning "foam" and "noose," respectively, may serve as a suitable specific epithet for the fungus.

Dactylella aphrobrocha sp. nov.

Mycelium sparsum; hyphis hyalinis, septatis, plerumque 1.7–3.7 μ crassis, hic illic ex ramulis bilocularibus (raro trilocularibus) vulgo 11–28 μ longis et 2.4–4.7 μ crassis laqueos circulares 20–38 μ latos proferentibus qui in 3 cellulis arcuatis consistunt et foramen rotundum vel rotundo-triangulum 12–27 μ latum circumdant; cellulis arcuatis 15–37 μ longis, medio 4–8.6 μ crassis, extremo 2.4–4.7 μ crassis, denso eorum protoplasmate circum lacunam centralem elongatam globuliferam disposito; vermiculo nematoideo in laqueum apertum introito omnibus tribus cellulis abrupte se contrahentibus, animal magnopere comprimentibus, id ita necantibus, integumentum ejus perforantibus, hyphas intus evolventibus quae carnem exhauriunt; hyphis assumentibus mox mediocriter septatis, saepius plus minusve ramosis, maximam partem 2–5 μ crassis sed interdum in cellulis usque 7 μ crassis abeuntibus, quandoque inter se conjunctis. Hyphae fertiles incoloratae, erectae, saepe 450–525 μ altae, basi 6–7 μ crassae, sursum leniter attenuatae, apice circa 2 μ crassae, ibi unum conidium ferentes, quandoque ramo brevi subter apicem praeditae denique aliud conidium gignentes; conidiis incoloratis, plerumque late fusiformibus, 2–4 septatis, saepissime quadriseptatis, tum plerumque ex toto 41–55 μ (saepius circa 46.7 μ) longis et 17–26 μ (saepius circa 21.9 μ) crassis, cellula infima 4.2–9.1 μ (saepius circa 6.2 μ) longa, cellula secunda 3.2–7.6 μ (saepius circa 5 μ) longa, cellula media 22.8–34 μ (saepius circa 27.3 μ) longa, cellula paenultima 2.6–6.5 μ (saepius circa 4.1 μ) longa, cellula summa 1.8-6 μ (saepius circa 4.1 μ) longa; cellula secunda interdum etiam rarius cellula paenultima appendicem filiformem ad pares angulos emittente, appendice incolorata, recta vel curvata, continua vel 1–2 septata, 40–300 μ longa, basi 1.8–2.5 μ crassa, sursum attenuata, apice circa 0.8 μ crassa.

Vermiculos nematoideos varios capiens consumensque habitat in humo silvestri prope Greensboro, North Carolina.

Mycelium scanty, spreading; vegetative hyphae colorless, septate, mostly 1.7 to 3.7 μ wide, often, especially in presence of nematodes, giving rise on curving stalks to circular rings in usually perpendicular positions; the stalks, commonly 11 to 28 μ long and 2.4 to 4.7 μ wide, consisting usually of two cells but occasionally composed of three cells; the rings, commonly 20 to 38 μ in outside diameter and surrounding a circular or rounded triangular aperture 12 to 27 μ wide, being consistently composed of three arcuate segments; the arcuate cells usually 15 to 37 μ long, 4 to 8.6 μ wide in the middle and 2.4 to 4.7 μ wide at the ends, containing dense homogeneous protoplasm in a parietal layer surrounding a central elongated lacuna filled with numerous globules—the first and third of the cells being united to one another as well as to the distal end of the stalk, and all the cells lacking a median protrusion on the inner side; on entrance of a nematode into the aperture the arcuate cells contracting abruptly, all three indenting the animal broadly and deeply, thereby strongly constricting it and soon disabling it, then perforating its integument to extend lengthwise through its body assimilative hyphae that appropriate the fleshy contents; the assimilative hyphae for the most part 2 to 5 μ wide but sometimes terminating in cells up to 7 μ in width. Conidiophores colorless, erect, often 450 to 525 μ high, 6 to 7 μ wide at the base, tapering gradually upward, about 2 μ wide at the tip, there bearing a single conidium, and sometimes bearing another conidium on a short branch given off a little below the tip. Conidia colorless, usually broadly spindle-shaped, sometimes 2- or 3-septate, but most often divided by four cross-walls in such wise that the middle cell is much larger than the others—then mostly 41 to 55 μ (average 46.7 μ) long and 17 to 26 μ (average 21.9 μ) wide, with the basal cell measuring often 4.2 to 9.1 μ (average 6.2 μ) in length, the parabasal cell 3.2 to 7.6 μ (average 5 μ), the median cell 22.8 to 34 μ (average 27.3 μ), the penultimate cell 2.6 to 6.5 μ (average 4.1 μ), and the apical cell 1.8 to 6 μ (average 4.1 μ) ; the small parabasal cell, and occasionally also the small penultimate cell, under some conditions putting forth at a right angle with the spore axis an aerial hyphal appendage; the appendage being colorless, straight or flexuous, 40 to 300 μ long, 1.8 to 2.5 μ wide at the base, tapering gradually to a width of 0.8 μ at the tip, continuous or divided in the broader proximal portion by one or two cross-walls.

Capturing eelworms of different species it occurs in leaf mold in woods near Greensboro, North Carolina.

A DACTYLELLA WITH ADHESIVE COLUMNAR OUTGROWTHS THAT SOMETIMES UNITE TO FORM IRREGULAR MESHES

In some maizemeal-agar plate cultures which after having been overgrown with *Pythium* mycelium had been further planted with leaf mold collected in deciduous woods near Butternut, Wisconsin, in September 1938, there appeared among various other nematode-capturing hyphomycetes a *Dactylella* that in a general way resembled *D. gephyropaga,* yet with respect to several phases of morphology differed rather markedly from that species. Its mycelial hyphae bore columnar outgrowths that not infrequently were composed of three or more cells (FIG. 3, *J, a, b*), though most often they consisted of one or two cells (FIG. 3, *K, a, b; L, a*). These outgrowths, like those of *D. gephyropaga,* were operative in capturing eelworms through adhesion (FIG. 3, *K, c; L, b–d*), the captives soon being disabled from intrusion of globose infective bodies and then undergoing invasion by assimilative hyphae. The fungus produced conidia which usually contained three (FIG. 3, *M–O*) or four (FIG. 3, *P, Q*) cross-walls, but were noticeably longer than the triseptate and quadriseptate conidia characteristic of *D. gephyropaga.* After falling on a moist substratum the conidia often germinated in a commonplace manner by putting forth a germ hypha from each end (FIG. 3, *R*).

The same fungus came to light again a few years later in maizemeal-agar plate cultures which after being overgrown with *Pythium irregulare* Buism. had been further planted through addition of small quantities of leaf mold gathered on October 2, 1941, near Presque Isle, Maine, in woods consisting largely of poplar (*Populus* sp.) and beech (*Fagus grandifolia* Ehrb.) trees. More recently it reappeared in more than a dozen maizemeal-agar plate cultures which after being permeated with *Pythium* mycelium had been planted with small quantities of wheat (*Triticum aestivum* L.) straw kindly collected by Dr. W. J. Zaumeyer near Hermiston, Oregon, on August 20, 1947. In these later cultures the distinctive characteristics of the fungus were brought more clearly into relief through the circumstance that nearly at the same time wholly typical material of *Dactylella gephyropaga* was found developing abundantly in nematode-infested agar plate cultures planted with leaf mold collected near Greensboro, North Carolina, and near Farmer, North Carolina, late in December, 1947. On transferring conidia aseptically from erect sporophores to tubes of sterile maizemeal agar the Oregon hyphomycete was obtained in pure culture. Comparison with pure cultures of *D. gephyropaga* originating from the two aforementioned localities in North Carolina as well as from several localities in Maryland and Virginia revealed it as a separate species of the same genus. Since the new *Dactylella* here in question has so far been obtained only from northern localities, and has never been recognized in the numerous cultures which in the course of 15 years were prepared with decaying detritus from many different places near Washington, D. C., there is reason to suspect that it may be distributed in colder regions than *D. gephyropaga*.

In the laboratory, certainly, the new fungus shows adaptation to lower temperatures than *Dactylella gephyropaga*. When portions of agar newly permeated with its mycelium are excised from pure cultures and placed on nematode-infested substratum kept at temperatures between 25° and 30° C., scarcely any hyphae are extended from the transferred mycelium, and virtually no eelworms will be captured, though within this temperature range under like conditions *D. gephyropaga* promptly sends out mycelial hyphae in all directions, and on them produces columnar outgrowths and scalariform networks that operate with spectacular efficiency in the capture of nematodes. When similar preparations are kept at temperatures between 15° and 18° C., however, the two fungi show reversed capacity for predacious activity; the new species soon extending many mycelial filaments well beset with columnar outgrowths effective in capturing eelworms, whereas *D. gephyropaga* will remain almost wholly inactive. Owing to its different temperature adaptations and its departures in morphology the new *Dactylella* compares with *D. gephyropaga* in much the same way as my *Dactylaria psychrophila* (**16**: 154–163) compares with the apparently more widespread retiary hyphomycete I described earlier under the binomial *Dactylaria thaumasia* (**12**: 518–523).

Unlike *Dactylella gephyropaga*, the Oregon strain of the new *Dactylella* (FIG. 6; FIG. 7), as also the strains from northern Wisconsin and northern Maine, often gives rise to predacious organs about as freely when growing undisturbed in pure culture on maize-meal agar (FIG. 6, *A–F*; FIG. 7, *A–C*) as when developing on nematode-infested substratum. In either type of culture some of the predacious organs consist individually of a short, stout, dome-shaped or columnar unicellular outgrowth filled uniformly with dense protoplasm of finely granular texture (FIG. 6, *A*; *B*; *C, a*; *E, a*. FIG. 7, *B, a*). The columnar outgrowths of somewhat greater length are commonly pluricellular, consisting of two (FIG. 6, *C, b, c*; *E, b*. FIG. 7, *A, a*; *B, b*; *D, a*), three (FIG. 7, *A, b*), four (FIG. 6, *D, a*; *E, c–e*), or more (FIG. 6, *F*) cells. Here and there two bicellular outgrowths may be found united distally by a unicellular bridging segment (FIG. 6, *C, d*) to form a rectangular mesh similar in shape and cellular make-up to the meshes of *Dactylella gephyropaga*. In addition, meshes of rectangular shape occur that show only little departure from the design characteristic of *D. gephyropaga* in having the columnar outgrowths united distally by a two-celled (FIG. 7, *B, c*) rather than by a one-celled bridging connection. On the whole, however, union between columnar outgrowths is much less frequent than in *D. gephyropaga*, and takes place in a more haphazard manner so that for the most part meshes are formed singly in promiscuously scattered positions, with comparatively little uniformity of size, shape, or cellular composition (FIG. 6, *D, b*,

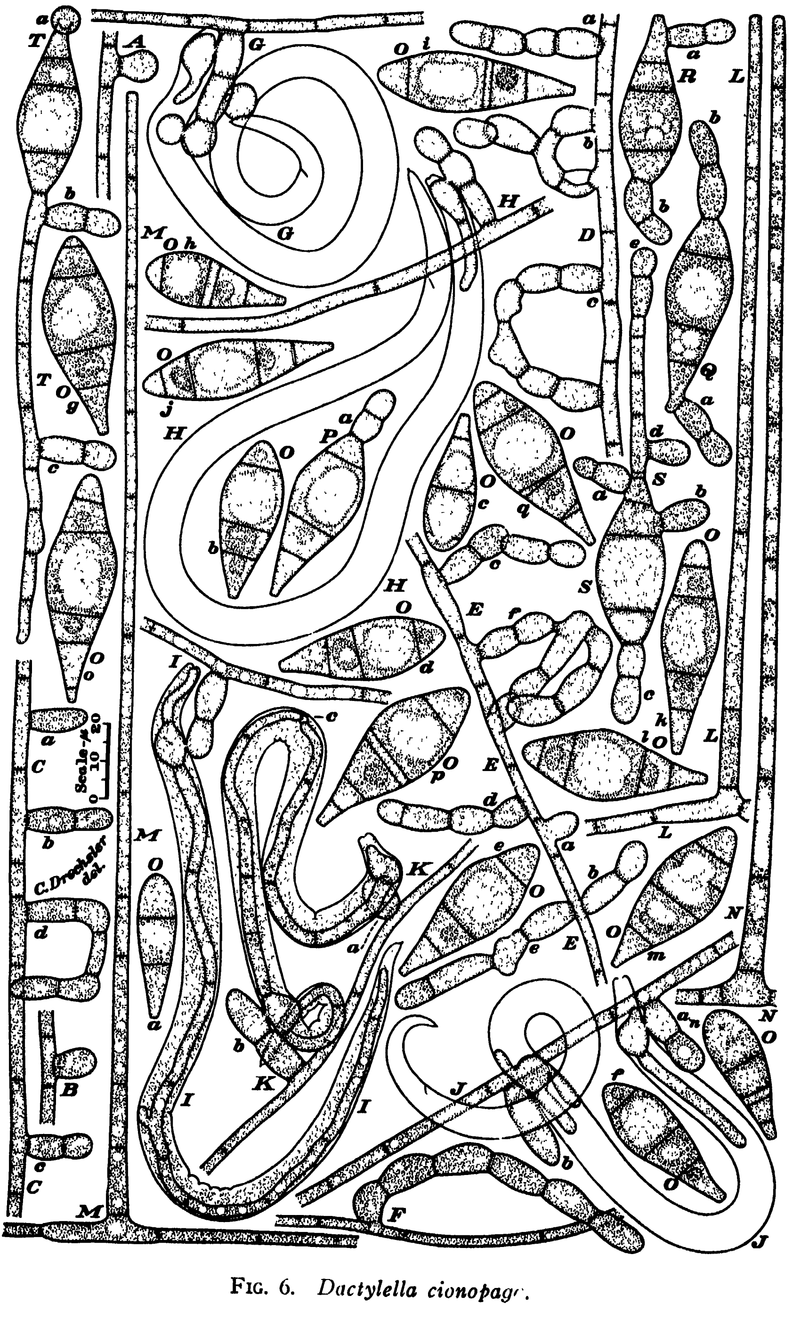

FIG. 6. *Dactylella cionopaga*.

c; E, f. FIG. *7, C*). The arrangement of numerous meshes into extended scalariform networks, so frequent in *D. gephyropaga,* is not characteristic of the present fungus.

Since the new species forms closed meshes in lesser number than *Dactylella gephyropaga,* its capture of nematodes is accomplished almost wholly through adhesion. Very frequently only a single outgrowth is operative in holding an eelworm (FIG. 6, *G–I;* FIG. *7, E–H*), though often, again, two or more outgrowths (FIG. 6, *J, a, b; K, a, b.* FIG. *7, D, b, c*) are found participating. No coating of adhesive material is to be seen on the undisturbed outgrowth, but after a struggling animal has been held fast for some time a sizable cushion of colorless glutinous substance often becomes visible, especially in instances where the contact between fungus and eelworm is conveniently presented in profile view (FIG. 6, *K, b;* FIG. *7, E*). Soon after capture is effected the animal's integument is narrowly perforated by one or more slender protrusions put forth by the fungus. Where pluricellular outgrowths are operative a protrusion may be extended from any or all of the component segments. Thus in the case of bicellular outgrowths, sometimes the distal segment (FIG. 6, *I; K, b.* FIG. *7, D, c; E*) and sometimes the proximal segment (FIG. 6, *J, a, b;* FIG. *7, F, G*) is directly active in penetrating into the captive; while with three-celled outgrowths penetration may take place from the median segment (FIG. 6, *H*), from the basal and apical segments (FIG. *7, H*), or from all segments (FIG. 6, *G*). Once the integument is penetrated the narrow protrusion gives rise within the fleshy interior to a globose infective body (FIG. 6, *G*) that continues to enlarge until it occupies the entire width of the animal and in some instances may even distend the integument noticeably (FIG. 6, *K, b;* FIG. *7, G*). The globose infective body, as in many other nematode-capturing hyphomycetes, disables the animal, and thereupon puts forth assimilative hyphae (FIG. 6, *H, J;* FIG. *7, E, H*) which invade the helpless captive lengthwise (FIG. 6, *F*) until it is occupied from head to tail (FIG. 6, *I, K;* FIG. *7, D*). Where the captured eelworm is very slender, like the eelworms that abundantly infested the plate cultures planted with the Oregon wheat straw (FIG. 6, *G–K;* FIG. *7, D, F–H*), only a single assimilative hypha may be developed in most portions of the fleshy body, though stouter animals usually become permeated

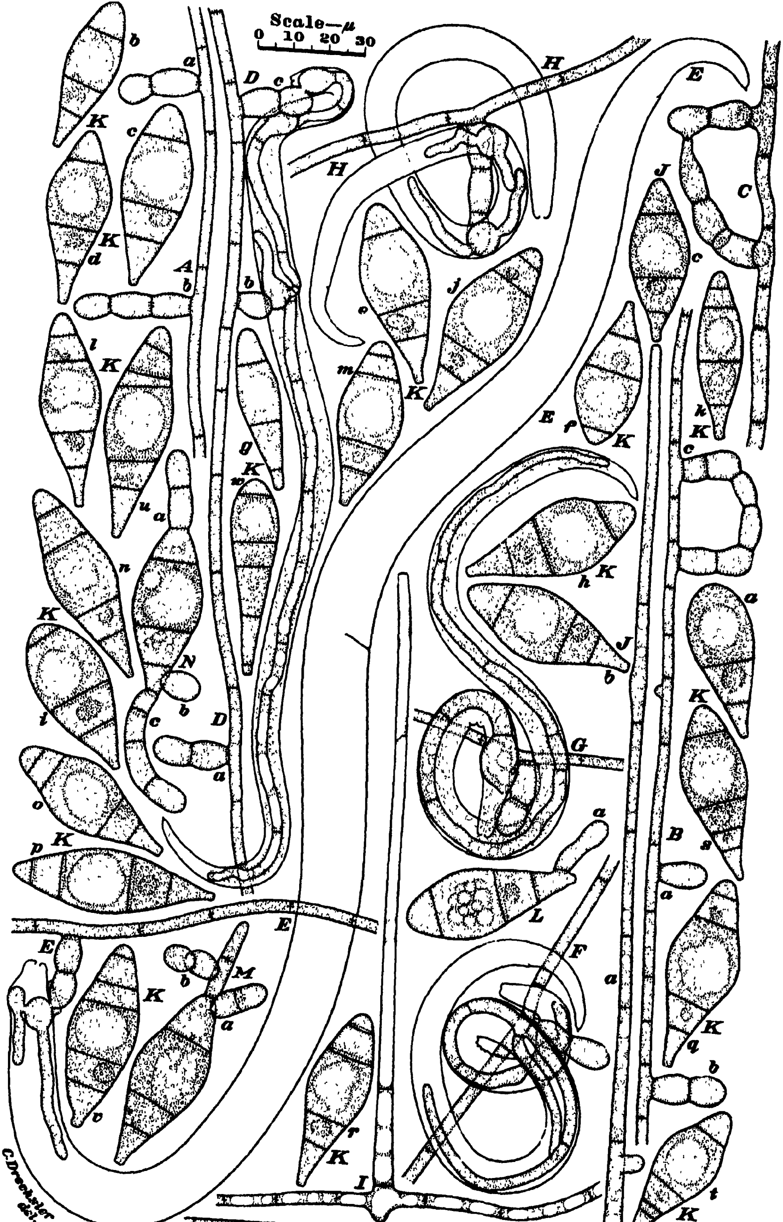

FIG. 7. *Dactylella cionopaga.*

by assimilative hyphae in numbers from two to six. Assimilative filaments of separate origin, and in stout eelworms assimilative hyphae arising from the same infective body, often become united distally through vegetative fusion (FIG. 6, *K, c*). After they have largely absorbed the globulose degenerating contents of the nematode, the assimilative hyphae show an increasingly vacuolate condition (FIG. 7, *D*). Ultimately as the animal becomes wholly depleted of digestible material the assimilative hyphae, through continued withdrawal of protoplasm backward into the external mycelium, are emptied of all living contents, and their membranous envelopes, together with the collapsing integument of the eelworm, gradually vanish from sight.

The fungus sporulates readily both in pure culture and on nematode-infested substratum. Its erect conidiophores (FIG. 6, *L–N*; FIG. 7, *I*) commonly vary from 170 to 300 μ in height, and thus seem generally shorter than those of *Dactylella gephyropaga*, as well as of most other similarly robust nematode-capturing hyphomycetes. Often a conidiophore (FIG. 7, *J, a*) after forming one conidium terminally (FIG. 7, *J, b*) will resume growth to produce a second conidium (FIG. 7, *J, c*) on a new tip farther upward; the first conidium being thereby pushed sideways and then presenting much the appearance of having been formed laterally. Occasionally growth is resumed once again, with ensuing development of a third conidium above the second, which in the meantime has like the first been brought into a lateral position.

The conidia thus formed, while of about the same width as those of *Dactylella gephyropaga*, are of noticeably greater length, and consequently have a somewhat more elongated shape together with greater volume. At the distal end they usually are broadly rounded, whereas proximally they taper toward a bluntly truncate base; so that they show various intergradations from a broadly fusiform to a clavate shape. In their septation they vary more than the conidia of *D. gephyropaga*, or of *D. aphrobrocha* and *D. bembicodes* among related constricting species. Many of the smaller, usually clavate specimens contain only two cross-walls (FIG. 6, *O, a*; FIG. 7, *K, a*). Among well developed conidia individuals with three (FIG. 6, *O, b–f*; FIG. 7, *K, b–i*) or four (FIG. 6, *O, g–n*; FIG. 7, *K, j–t*) cross-walls predominate, though not infrequently a five-septate condition (FIG. 6, *O, o, p;* FIG. 7, *K, u–w*), virtually unknown in *D. gephyropaga* as also in *D. aphrobrocha,* and even a six-septate condition (FIG. 6, *O, q*) is encountered here. In quadriseptate specimens the cross-walls most commonly are placed in symmetrical arrangement (FIG. 6, *O, g, j, k, l;* FIG. 7, *K, j–r*) with a large cell occupying a median position between two smaller distal (apical and penultimate) cells and two smaller proximal (basal and parabasal) cells, yet rather often they occur in unsymmetrical arrangements with the large cell placed in penultimate (FIG. 6, *O, h, i;* FIG. 7, *K, s, t*) or in terminal (FIG. 6, *O, n*) position. The large cell of mature conidia contains usually a big conspicuous vacuole, while one or two of the remaining cells reveal commonly a globose mass that seems to be composed of densely conglomerated coarse granules. In some conidia where the large cell apparently begins to undergo division after its big vacuole has been formed, the belated cross-wall is laid down only within the parietal protoplasmic layer, leaving a large opening through which the vacuole extends from one daughter cell to the other (FIG. 6, *O, c, m*). Here and there a septum may be found that does not extend entirely across the conidium, but cuts off merely a small wedge-like portion from the proximal or the distal end of one of the larger cells (FIG. 6, *O, q.*)

Instead of germinating in commonplace manner by emission of a germ hypha from each end, many conidia after falling on a moist substratum give rise directly to adhesive outgrowths. Most frequently these outgrowths are put forth from one or from both of the end cells (FIG. 6, *P, a; Q, a, b; R, a, b; S, a, c; T, a.* FIG. 7, *L, a; M, a; N, a–c*), though sometimes they are found arising from intermediate cells (FIG. 6, *S, b*). Often, again, a conidium gives rise to one or more adhesive outgrowths (FIG. 6, *S, d, e; T, b, c.* FIG. 7, *M, b*) on a stout germ hypha. Such germ hyphae, which in themselves seem to lack adhesiveness, consist as a rule of only a few rather short segments, yet occasionally they may attain a length in excess of 100 μ (FIG. 6, *T*). While the adhesive outgrowths produced by detached conidia, like those arising from mycelial hyphae, commonly consist of one or two cells, in more than a few instances they are composed of three or four segments (FIG. 7, *N, c*).

In view of the frequently columnar shape of the predacious outgrowths a term compounded of two words meaning "pillar" and "trap," respectively, is proposed as specific epithet for the fungus.

Dactylella cionopaga sp. nov.

Mycelium sparsum; hyphis incoloratis, ramosis, mediocriter septatis, plerumque 2–5 μ crassis, hic illic prominentia glutinosa ferentibus; prominentibus glutinosis quandoque tuberiformibus sed saepius columnaribus, simplicibus vel parce ramosis, plerumque 10–90 μ longis, 5.5–10.5 μ crassis, in 1–7 (saepius in 1 vel 2) cellulis protoplasmate dense granuloso farctis consistentibus, ad septa leviter constrictis, quandoque inter se inordinatim conjunctis, rarius in laqueos quadrilateros vel semicirculos connexis—his prominentibus laqueisque vermiculos nematoideos errantes tenentibus, deinde tum integumentum animalis captivi perforantibus, tuber mortiferum intrudentibus, hyphas assumentes evolventibus quae carnem exhauriunt; hyphis assumentibus plerumque 2–5 μ crassis, mediocriter septatis. Hyphae fertiles incoloratae, erectae, vulgo simplices, 2–11 septatae, plerumque 170–300 μ altae, basi 5–7 μ crassae, sursum leniter attenuatae, apice saepe circa 3 μ crassae, ibi unum conidium ferentes, interdum recrescentes et 1 vel 2 alia conidia gignentes; conidiis incoloratis, plerumque late fusiformibus vel aliquid clavatis, apice late rotundatis, basi truncatis, vulgo 35–60 μ longis, 13–21 μ latis, 2–6 septatis plerumque triseptatis et quadriseptatis, post disjunctionem prominentia glutinosa praecipue ex apice et ex basi atque ex hypha germinationis brevi saepe emittentibus.

Vermiculos nematoideos diversos capiens consumensque habitat in stramento Tritici aestivi putrescenti prope Hermiston, Oregon, atque in humo silvestri prope Butternut, Wisconsin, et prope Presque Isle, Maine.

Mycelium scanty, spreading; vegetative hyphae colorless, septate, mostly 2 to 5 μ wide, here and there giving rise to adhesive outgrowths; adhesive outgrowths sometimes tuberiform but more often columnar, simple or branched, mostly 10 to 90 μ long, 5.5 to 10.5 μ wide, composed of 1 to 7 (usually of 1 or 2) cells densely filled with finely granular protoplasm, noticeably constricted at the septa, sometimes becoming fused with one another and thereby forming meshes which occasionally are rectangular or semicircular but more usually are of irregular shape—these outgrowths and meshes capturing nematodes through adhesion, later narrowly perforating the integument of each captured animal, then intruding a globose infective body and extending assimilative hyphae lengthwise through the interior to appropriate the fleshy contents; the assimilative hyphae 2 to 5 μ wide, septate at moderate intervals. Conidiophores colorless, erect, commonly unbranched, eventually divided by 2 to 11 cross-walls, mostly 170 to 300 μ high, 5 to 7 μ wide at the base, gradually tapering upward, about 3 μ wide at the tip, there producing a single conidium, though sometimes after repeated elongation forming one or two additional conidia. Conidia colorless, mostly broadly spindle-shaped or somewhat clavate, broadly rounded at the distal end but at the narrower proximal end tapering toward the bluntly truncate base, commonly 35 to 60 μ long, 13 to 21 μ wide, containing 2 to 6 cross-walls though most usually triseptate or quadriseptate, often germinating by putting forth adhesive outgrowths especially from the basal and apical segments, though sometimes from an intermediate segment or from a short non-adhesive germ hypha.

Capturing and consuming different species of nematodes it occurs in decaying culms of *Triticum aestivum* near Hermiston, Oregon, and also in leaf mold from deciduous woods near Butternut, Wisconsin, and near Presque Isle, Maine.

A RETIARY DACTYLARIA PRODUCING TRISEPTATE CONIDIA FREQUENTLY OF UNUSUAL WIDTH

Agar plate cultures prepared, as opportunity offered, with small quantities of decaying material found on plant specimens received from Florida and Louisiana during the last 15 years have brought to light many of the nematode-capturing fungi that occur everywhere in the region surrounding Washington, D. C. In addition to *Arthrobotrys oligospora*, *A. conoides*, and *A. musiformis* Drechsler (12: 477–482) among the hyphomycetes employing adhesive networks for capturing eelworms, these plate cultures often displayed abundant development ot predacious and reproductive apparatus wholly typical of *Dactylaria thaumasia*. Now and then they showed, further, a retiary form that arrested attention because many of its broad triseptate conidia appeared to be considerably wider than those of *D. thaumasia*. As the plate cultures were nearly always overrun by mites at an early stage, owing to heavy infestation of these animals in material from southern localities, they gave little encouragement for closer study or for isolation of the aberrant form. Better conditions for appropriate treatment were eventually given when presumably the same fungus developed rather extensively in several maizemeal-agar plate cultures which after being overgrown with mycelium of *Pythium ultimum* Trow had been further planted by adding small quantities of leaf mold gathered in deciduous woods near Roanoke, Virginia, on October 11, 1946. From observations on both pure and nematode-infested cultures the

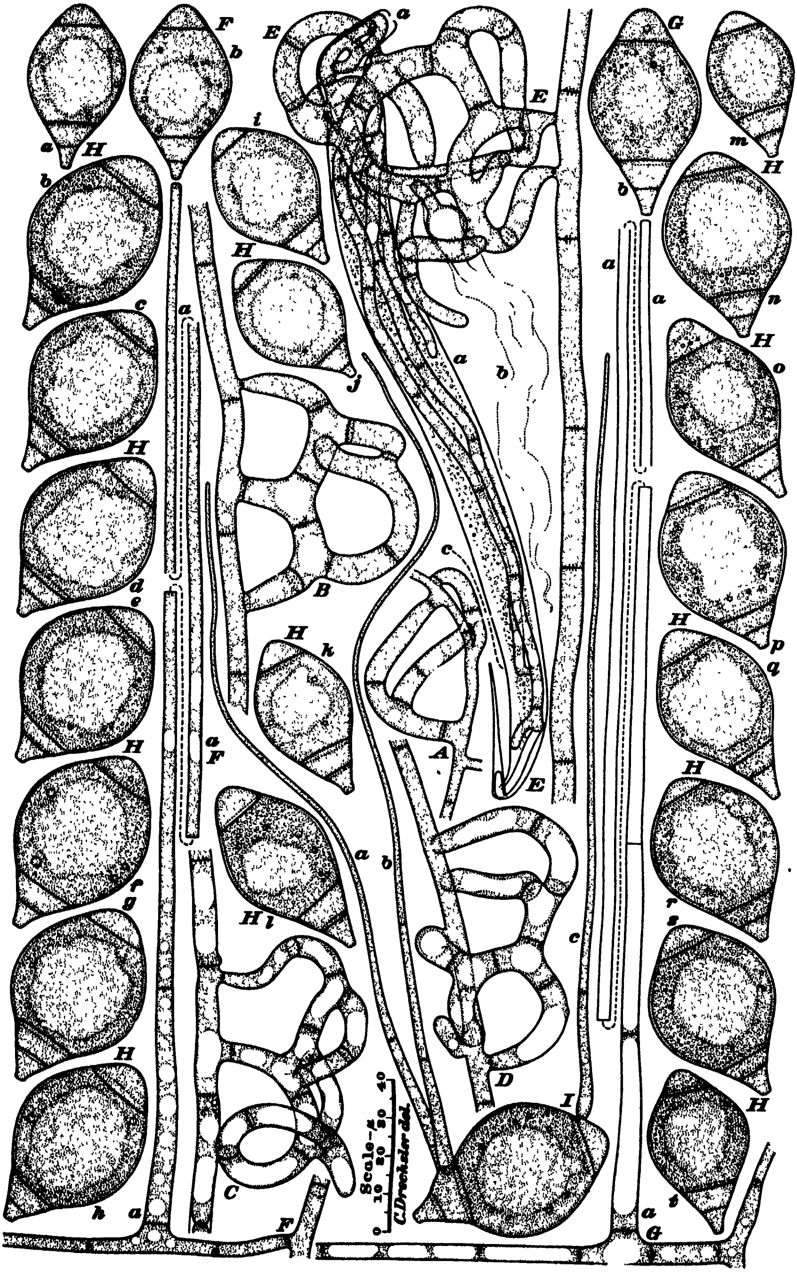

FIG. 8. *Dactylaria eudermata.*

fungus here concerned would seem to differ sufficiently from *D. thaumasia* to merit recognition as a separate species.

When sizable portions of agar well permeated with young mycelium are excised from pure cultures of the new hyphomycete and placed on agar plate cultures well infested with eelworms of such genera as *Rhabditis, Panagrolaimus, Acrobeloides*, and *Plectus,* new hyphae are extended in all directions from the transferred mycelium into the surrounding substratum. These radiating hyphae give rise at varying intervals to three-dimensional networks (FIG. 8, *A–D;* FIG. 9, *A, B*) which like the similar networks in many allied species are produced through successive development of rather thick recurving branches (FIG. 9, *C, a*). Each of these branches forms a closed semicircular loop or bail-like element by fusing terminally, at some distance from its origin, with a small protuberance put forth by a neighboring hyphal segment. The single fusion here takes place in much the same manner as each of the two fusions usually accomplished in the formation of a constricting ring. After the tips of the recurved branch and the opposed protuberance have come together somewhat broadly (FIG. 9, *C, b*) the portions of membrane at the surface of contact dissolve away (FIG. 9, *C, c*), though some time later the resulting continuity is modified by deposition of a cross-wall near the place of union (FIG. 9, *C, d*).

The hyphal bails and the networks compounded from them operate much like the similar apparatus employed by various other nematode-capturing hyphomycetes. Eelworms that in their continuous movement happen to run afoul of these structures are held fast despite their energetic struggles to escape. In some instances where little hyphal enwrapment is to be seen (FIG. 10, *A*) the captured animal must obviously be held mainly if not wholly through adhesion; but in other instances encirclement of the animal in one or several meshes (FIG. 9, *D*) gives reason for belief that it is held through both adhesion and entanglement. Following narrow perforation of the integument, the fungus disables the struggling eelworm by intruding one (FIG. 10, *A*) or more (FIG. 9, *D*) globose infective bodies from which assimilative hyphae are then extended (FIG. 9, *D*) to invade the fleshy interior from head to tail (FIG. 10, *B*). At first the assimilative hyphae are badly ob-

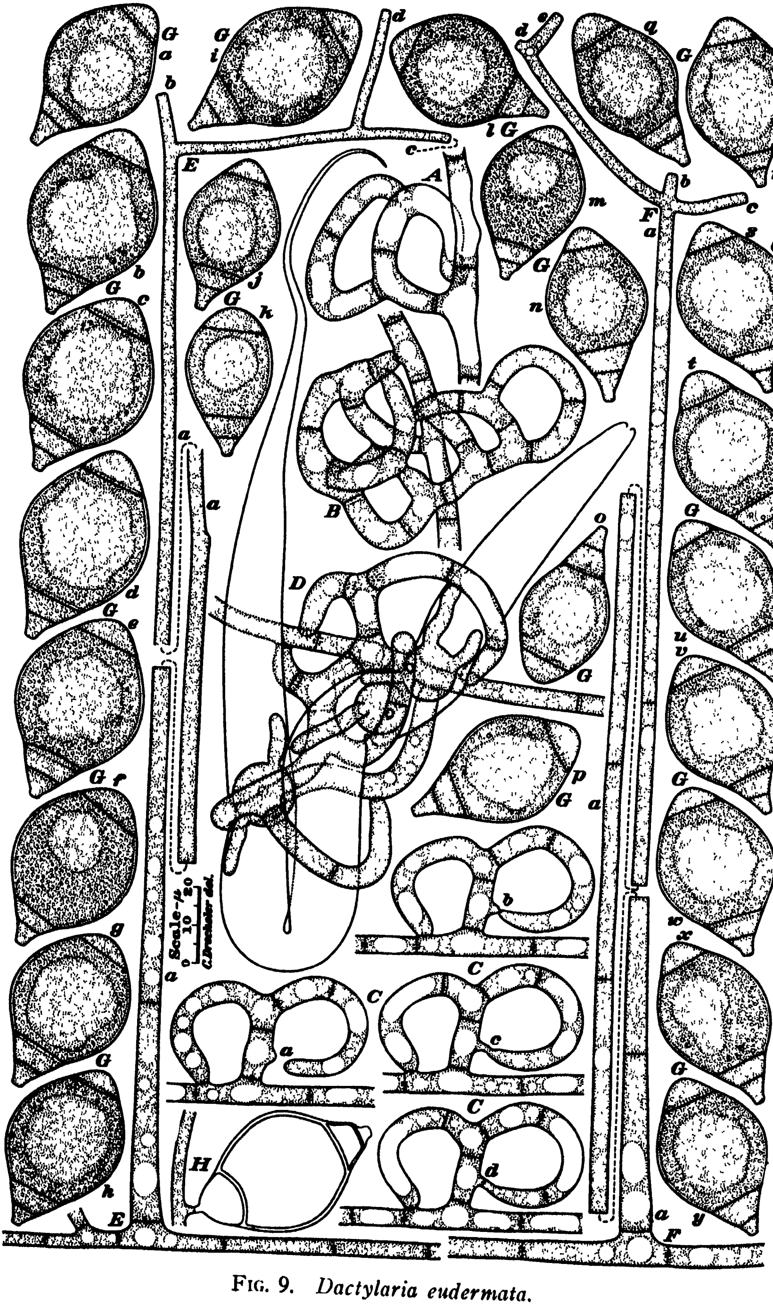

FIG. 9. *Dactylaria eudermata.*

scured from view owing to the globulose degeneration of the animal's musculature and organs. Later when the degenerating material has been largely absorbed (FIG. 8, *E, a*) the assimilative hyphae become more clearly visible, and instances of vegetative fusion between them are revealed (FIG. 8, *E, c*). At about this time they begin to show vacuoles. With further depletion of the digestible substance in the animal the vacuoles increase steadily in volume. Eventually the assimilative hyphae are wholly emptied of contents through withdrawal of their protoplasm backward into the external mycelium and, together with the collapsing pellicle, vanish from sight. In some instances the somewhat indurated infective body (FIG. 8, *E, b*) remains visible for a time after the associated hyphae have disappeared.

With ample nourishment being obtained through destruction of many nematodes the fungus soon produces conidiophores and conidia. The conidiophores arise in scattered positions from procumbent hyphae. Often they consist individually of a stout simple erect hypha, tapering gradually upward from base to tip, and ranging mostly from 0.4 to 0.5 mm. in height (FIG. 8, *F, a; G, a.* FIG. 10, *C, a*). These simple conidiophores bear terminally a single conidium (FIG. 8, *F, b; G, b.* FIG. 10, *C, b*) much like the simple conidiophores, for example, of *Dactylella aphrobrocha* and *D. bembicodes,* which, indeed, in their dimensions they resemble rather closely. The fungus likewise puts forth distally branched conidiophores (FIG. 9, *E, a; F, a*) that besides producing a spore at the tip of the main hypha (FIG. 9, *E, b; F, b*) bear additional spores singly on its primary branches (FIG. 9, *E, c; F, c, d*) and also on its secondary branches (FIG. 9, *E, d; F, e*) if such are present. As the branches are often rangy, sometimes exceeding 50 μ in length, the conidia produced plurally are in many instances attached at generous distances from one another. Nevertheless where conidia are held aloft in numbers of three or four they usually offer a clustered aspect, for owing to the large size of the individual spores the intervening spaces do not seem disproportionately wide.

The conidia produced by the fungus in nematode-infested culture (FIG. 8, *H, a–t;* FIG. 9, *G, a–y;* FIG. 10, *D, a–f*) owe their frequently large size more to their unusual width than to their length.

On the whole they appear about one-third wider and one-sixth longer than the similarly turbinate triseptate conidia of *Dactylaria thaumasia.* In fully developed spores the triseptate condition is so strongly predominant that the few biseptate specimens (FIG. 10, *D, f*) commonly present arouse misgivings as to their maturity, while quadriseptate conidia seem scarcely less alien to the fungus than to *Dactylella stenobrocha* and *Dactylella bembicodes.* As in *D. bembicodes* the three cross-walls are usually so placed that the penultimate cell greatly exceeds the others in dimensions and volume. This voluminous cell contains a large irregular vacuole, and is further distinguished by being surrounded with a noticeably thickened wall. When nematode-infested cultures, on being uncovered for microscopical examination, are exposed to dry air, the thin-walled apical cell collapses badly, whereas the sturdy penultimate cell, at least in respect to its peripheral outer membrane, commonly retains its shape; the result being that the conidia held aloft offer a curiously truncated appearance. Detached conidia are often found bearing one or more slender filamentous aerial outgrowths (FIG 8, *I, a–c*), sometimes over 200 μ long, which originate commonly from the parabasal and apical cells. Under somewhat humid and rather warm (25° to 35° C.) conditions such outgrowths are extended freely also from conidia still supported on the conidiophores. In water or on a moist nutrient substratum germination takes place by the emission of one or more submerged germ hyphae (FIG. 10, *E*). Fusion of germ hyphae with mycelial filaments is frequent and often leads to evacuation of all protoplasmic contents from the conidia concerned (FIG. 9, *H;* FIG. 10, *F*).

The fungus grows rapidly in pure culture on maizemeal agar. In test-tube cultures on this substratum it sometimes produces a more copious aerial mycelium than I have hitherto observed in cultures of any other nematode-capturing hyphomycete. Often the column of rather loose cottony growth immediately above an agar slant is surmounted by an upper layer of firm felt-like texture; the appearance given then being rather similar to that commonly presented by tube cultures of my *Pythium arrhenomanes.* A few days after a pure culture has been planted it begins to produce conidia, which for the most part, however, reveal only rather poorly the morphological features distinctive of conidia formed on nematode-infested substratum. Occasional specimens, it is true, are divided by three cross-walls into four cells whereof the one in penultimate position exceeds the others in size, but even in these specimens departure from the normal is often clearly evident in greatly reduced size of the apical and basal segments (FIG. 10, *G*, *a–c*). Very frequently the conidia formed in pure culture contain only two cross-walls, both of them placed near the proximal end, so that the large globose cell in terminal position here surmounts the much smaller parabasal and basal segments (FIG. 10, *G*, *d–m*). Frequently, again, the conidia contain only a single cross-wall, which being placed near the basal end delimits a large globose distal cell from a small proximal cell (FIG 10, *G*, *n–q*). Like the spores formed in nematode-infested culture those produced in pure culture are often found bearing one or more aerial outgrowths of slender filamentous shape (FIG. 10, *H*), or are provided with broader germ hyphae of ordinary vegetative character (FIG. 10, *I–K*). The germ hyphae here likewise often fuse with neighboring mycelial filaments (FIG. 10, *L*, *M*), thereby establishing a passageway through which the protoplasmic materials of the conidium may migrate into the mycelium.

Despite their frequently rather different appearance the conidia produced by the fungus in pure culture are not essentially different in kind from the conidia formed on nematode-infested substratum. They are manifestly not referable to a separate category of spores, such as is recognizable in the uniseptate allantoid conidia of *Dactylella heterospora* and in the globuliferous conidioid bodies of *D. doedycoides*. Their generally smaller size, the disproportionate reduction of their smaller cells, and more especially the usual occurrence of their large cell in distal position owing to frequent lack of a small cell at the apex, seem to come about as modifications resulting from the different nourishment supplied in maize-meal agar not inhabited by eelworms. It seems possible that the modifications in question fall outside the range of ordinary variation, and perhaps are to be construed as pathological abnormalities deriving from nutritional deficiency. In any case the tendency toward morphological deterioration here offers marked contrast

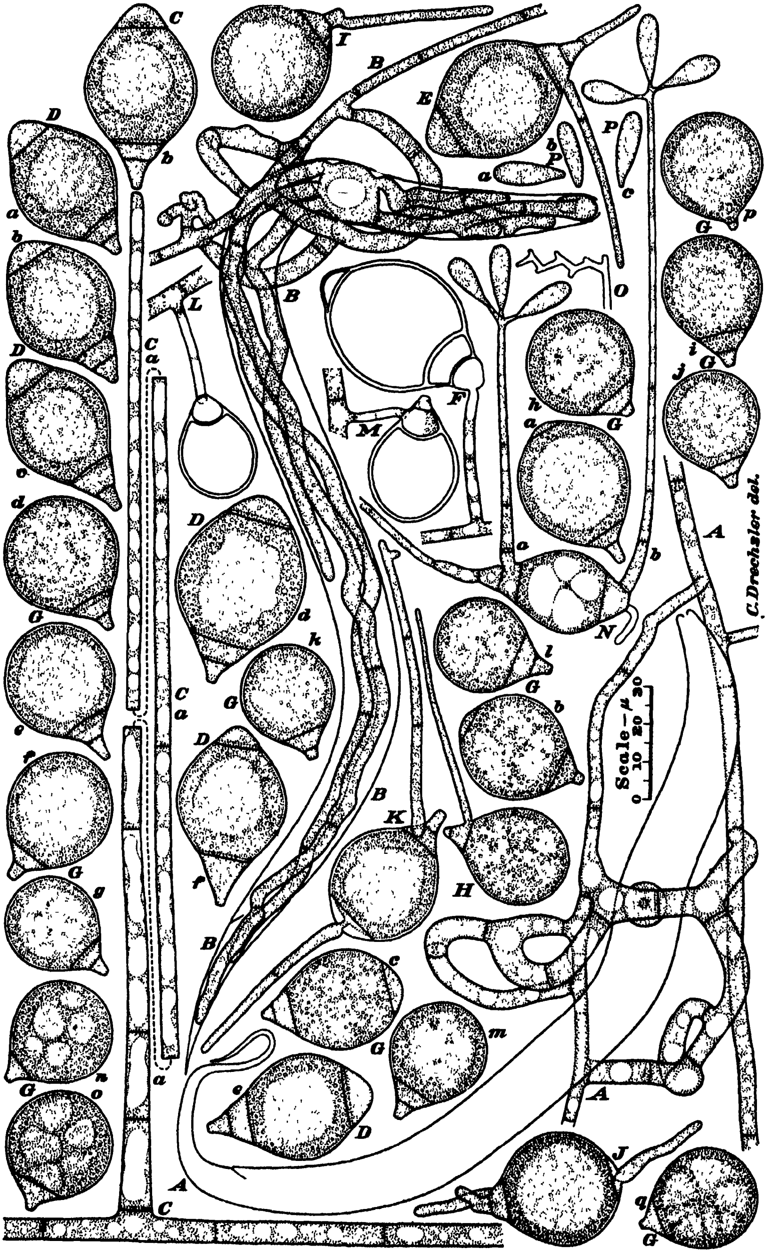

FIG. 10. *Dactylaria eudermata.*

with the luxuriant and wholly correct sporulation of *Dactylaria thaumasia* when grown in pure culture on maizemeal agar.

Yet conidia essentially different in kind from the broad triseptate spores hitherto discussed have come under observation. One of the original plate cultures in which the fungus had grown out directly from plantings of leaf mold showed within a limited area a number of detached triseptate conidia that after falling on the moist agar substratum had put forth one or more germ conidiophores (FIG. 10, *N*, *a*, *b*) bearing small secondary conidia in loose capitate arrangement. These germ conidiophores, measuring 65 to 275 μ in height and 3 to 4 μ in basal diameter, tapered gradually upward to a width of 1.5 to 2 μ at the tip, where they terminated usually in a few—mostly in 2 to 5—sterigmatic spurs each bearing a secondary conidium; though in some instances secondary conidia up to seven in number were found borne on spurs, each about 5 μ long, produced one after another by successive subapical branching (FIG. 10, *O*). The secondary conidia (FIG. 10, *P*, *a–c*) were colorless, unseptate, of clavate shape, 15 to 25 μ long, and 4.3 to 6.5 μ wide. In the same area of the Petri plate culture similar conidia were borne also on small conidiophores arising from mycelial filaments. Efforts to start pure cultures from the meager supply of unseptate conidia were unsuccessful, and the cultures started from broad triseptate conidia taken from tall conidiophores in the area have not so far yielded a secondary or regularly unicellular conidial stage. Although it seems probable that the subsidiary spore stage belonged in the life history of the present fungus, the connection has not yet been established. Much the same uncertainty remains here as was noted earlier (**16**: 163–166) in respect to the likely connection of a rather similar secondary conidial stage with *Dactylaria psychrophila*.

The fungus offers further parallelism with *Dactylaria psychrophila* in having a sporulating habit so meagerly capitate that its assignment to the genus *Dactylaria* needs to be justified in part by its manifestly close relationship to the more pronouncedly capitate *D. thaumasia*. As its adhesive meshes are of about the same dimensions as those of *D. thaumasia* and most other retiary hyphomycetes, its networks appear not to share the somewhat closer texture noted in the predacious apparatus of *D. psychro-*

phila; and certainly they do not share the markedly closer texture usual in networks of my *D. polycephala* (**12**: 527–531). Like *D. psychrophila* the fungus produces larger conidia that *D. thaumasia,* but its conidia owe their larger size mainly to greater width, whereas those of *D. psychrophila* owe their larger size mainly to greater length. Quadriseptate partitioning, so infrequent in conidia of the fungus, is commonplace among conidia of *D. psychrophila.* Pure cultures of the fungus on maizemeal agar have not so far shown chlamydospores or any other kind of indurated bodies; whereas similar cultures of *D. thaumasia* commonly produce thick-walled chlamydospores in such abundance that the substratum is given a reddish coloration.

A term meaning "with good, stout hide" may serve conveniently as specific epithet for the new hyphomycete in recalling the thick membrane surrounding the massive penultimate segment of its conidia.

Dactylaria eudermata sp. nov.

Mycelium effusum; hyphis sterilibus incoloratis, mediocriter septatis, plerumque 1.8–7.5 μ crassis, laqueos tenaces arcuatos vel circulares in reticula saepe conjunctos proferentibus; his laqueis reticulisque vermiculos nematoideos illaqueantibus, deinde tum integumentum animalis captivi anguste perforantibus, tuber mortiferum globosum intrudentibus, hyphas intus evolventibus quae carnem exhauriunt. Hyphae fertiles incoloratae, erectae, plerumque 2–8 septatae, vulgo 400–500 μ altae, basi saepius 6–9 μ crassae, sursum leniter attenuatae, apice 2.5–3.5 μ crassae, simplices vel prope apicem aliquid ramosae, itaque nunc unicum conidium gignentes, nunc 2–4 conidia in capitulum laxum ferentes; conidiis incoloratis, ellipsoideis vel obovoideis vel late turbineis, apice rotundatis, deorsum paulum attenuatis, basi truncatis, plerumque 37–55 μ (saepe circa 48 μ) longis, 21–35 μ (saepe circa 28 μ) crassis, vulgo triseptatis, paenultima eorum cellula 21–35 μ (saepe circa 29 μ) longa, aliis cellulis eorum multo minoribus, inter se subaequalibus, vulgo 4–8 μ (saepe circa 6 μ) longis; interdum omnibus cellulis glabris, interdum aliquibus cellulis minoribus 1 vel 2 appendicibus praeditis; appendicibus incoloratis, filiformibus, rectis vel flexuosis, continuis vel prope basim 1–2 septatis, plerumque 50–275 μ longis, basi 2–4 μ crassis, sursum leniter attenuatis, apice circa 1 μ crassis.

Vermiculos nematoideos diversos capiens consumensque habitat in humo silvestri prope Roanoke, Virginia.

Mycelium spreading; vegetative hyphae colorless, septate at moderate intervals, mostly 1.8 to 7.5 μ wide, often especially in the presence of nematodes giving rise to arched or circular hyphal meshes, which, though at first discrete, are later frequently compounded into more or less extensive networks; the meshes and networks capturing nematodes through adhesion and entanglement, then narrowly perforating the integument of each captured animal and intruding one or more infective bodies of subspherical shape, from which are extended assimilative hyphae, mostly 2 to 5 μ wide, to appropriate the fleshy contents. Conidiophores colorless, erect, usually containing 2 to 8 cross-walls, commonly 400 to 500 μ high, 6 to 9 μ wide at the base, tapering gradually upward to a width of 2.5 to 3.5μ near the tip, simple or somewhat branched at the distal end, consequently sometimes producing a single conidium and at other times bearing 2 to 4 conidia in loose capitate arrangement. Conidia colorless, ellipsoidal or obovoid or broadly turbinate, broadly rounded at the tip, somewhat tapered proximally, truncate at the base, mostly 37 to 55 μ (average about 48 μ) long, 21 to 35 μ (average about 28 μ) wide, commonly triseptate, the penultimate cell 21 to 35 μ (average about 29 μ) long, the other three cells much smaller, individually most often 4 to 8 μ (average about 6 μ) long and sometimes bearing 1 or 2 aerial outgrowths; aerial outgrowths colorless, filamentous, straight or flexuous, continuous or with 1 or 2 cross-walls in the proximal portion, 50 to 275 μ long, 2 to 4 μ wide at the base, tapering gradually upward to a width of approximately 1 μ at the tip.

Capturing and consuming nematodes of different species, it occurs in leaf mold in deciduous woods near Roanoke, Virginia.

A CANDIDA-LIKE DACTYLARIA CAPTURING EELWORMS BY ADHESION TO SLENDER-STALKED ADHESIVE KNOBS

Several maizemeal-agar plate cultures which after being permeated with mycelia of *Pythium mamillatum* Meurs had been further planted on April 29, 1948, with small quantities of partly decayed bluegrass (*Poa pratensis* L.) detritus newly collected in a field near Beltsville, Maryland, showed 16 days later some expanses of conidial apparatus rather similar to that of *Dactylaria candida* (Nees) Sacc., but differing in being less strongly capitate. A more decisive difference came to light when the underlying mycelium was examined, for though the vegetative hyphae manifestly obtained their nourishment by capture of nematodes abundantly infesting the substratum, the animals were in all instances held fast through adhesion to stalked globose unicellular knobs comparable in size and efficacy to the adhesive knobs utilized by my *Dactylella* *asthenopaga* (**12**: 496–499) and my *Dactylaria haptospora* (**13**: 456–461) as well as by the nematode-capturing fungus (**12**: 492–496) held referable to *Dactylella ellipsospora* Grove (22). The mycelium was wholly lacking in non-constricting rings, such as those employed very effectively by the widespread nematode-capturing hyphomycete I have assigned (**12**: 523–527) to the ancient species of Nees von Esenbeck. However, since this hyphomycete, besides producing non-constricting rings, gives rise terminally on very slender stalks to small globose unicellular knobs which in soft agar cultures have only rarely been found operative in capturing eelworms, it was necessary to consider whether the observed absence of non-constricting rings together with the greater size and efficiency of the globose knobs might perhaps have resulted from unusual environal conditions. The fungus growing out from the bluegrass detritus was therefore isolated by removing its conidia from the erect conidiophores to sterile agar. From the pure cultures thus obtained portions of agar well permeated with young mycelium were excised and placed on Petri plate cultures that had become infested with saprophilous nematodes introduced on decaying materials of various kinds. Among the cultures used in these trials were some that had been started by planting the sterile substratum with pieces of softened discolored roots of matai or Chinese waternut [*Eleocharis dulcis* (Burm. f.) Henschel] plants received from Winter Park, Florida, late in July, 1948. After being promptly overgrown by my *Pythium myriotylum* the agar here afforded abundant multiplication of a slender species of *Panagrolaimus*, which later suffered destruction in large numbers when young mycelium of the Beltsville hyphomycete was superadded. As the invasive development of predacious and parasitic fungi is in general more clearly visible in slender than in stout eelworms, material pertaining to or resulting from the destruction of the *Panagrolaimus* was used advantageously in preparing the figures relating to the fungus (FIGS. 11, 12).

None of the nematode-infested cultures in which portions of young mycelium were planted showed any development of non-constricting rings. From the superadded mycelium rather slender filaments grew out that at somewhat variable intervals and mostly in submerged positions consistently gave rise to unicellular adhesive

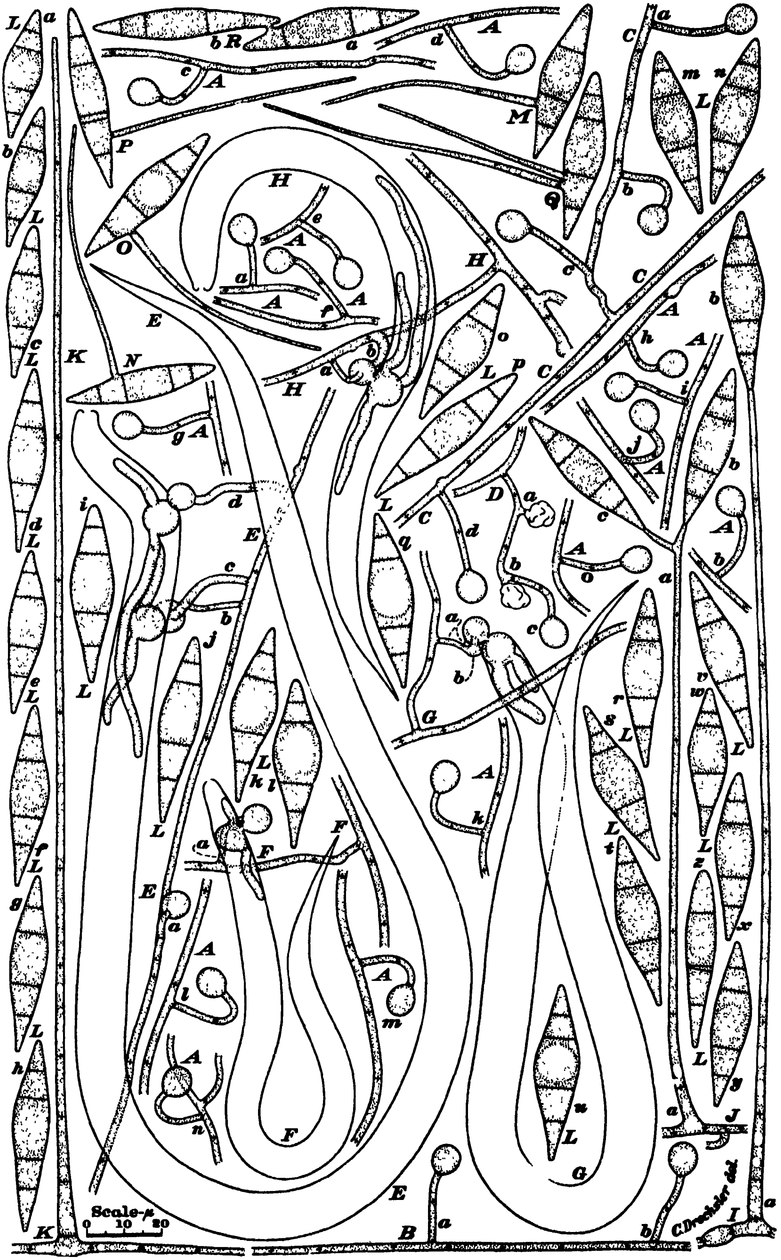

FIG. 11. *Dactylaria haptotyla.*

knobs on stalks sometimes consisting of one cell (FIG. 11, *A, a, b.* FIG. 12, *A, a–d; D, a*) but more often consisting of two cells (FIG. 11, *A, c–o; B, a, b; C, a–d; E, a.* FIG. 12, *A, e–k; B, a, b; C, b*) and occasionally consisting of three cells (FIG. 12, *C, c*). Most frequently the stalks were 1.4 to 1.9 µ wide and from 10 to 25 µ long. They appear appreciably stouter, therefore, than the corresponding stalks of *Dactylaria candida,* which, as a rule, measure only 1 to 1.4 µ in width. At the same time they seem markedly more slender as well as generally longer than the homologous stalks of *Dactylella ellipsospora,* since these usually measure 2.4 to 3 µ in width and 5 to 10 µ in length; the dimensional contrast here offering some parallelism with the contrast between the stalks supporting the constricting rings of *Dactylella aphrobrocha* and those supporting the constricting rings of *Dactylella bembicodes.* Nearly the same degree of contrast is evident when comparison is extended to the stalks of *Dactylella asthenopaga,* which, as is apparent from their recorded dimensional ranges—2 to 3 µ for width, and 3 to 10 µ for length—are similarly of short sturdy conformation. The stalks of *Dactylaria haptospora,* commonly 1.5 to 2.5 µ wide and 4 to 30 µ long, offer notably better agreement with respect to measurements.

The globose or prolate ellipsoidal adhesive knobs of the Beltsville fungus have been found varying mostly from 7 to 10 µ in length and from 6 to 8.5 µ in width. As has been intimated they differ little in size from the adhesive knobs of the three previously described nematode-capturing hyphomycetes that produce no other type of predacious organ; though on a strict comparison of relevant measurements they would seem slightly smaller than the knobs of *Dactylella ellipsospora,* and slightly larger than those of *Dactylella asthenopaga* and *Dactylella haptospora.* They are markedly larger, of course, than the meagerly operative knobs of *Dactylaria candida,* which commonly measure only 4 to 7 µ in length and 3.8 to 6 µ in width. In nearly equal measure they exceed in size also the meagerly operative knobs of my *Dactylella lysipaga* that usually range from 5 to 8 µ in length, and from 4.5 to 6 µ in width.

Frequently in predacious organs that have attained some age without capturing a nematode, the adhesive cell loses its protoplasmic contents and collapses emptily (FIG. 11, *D, a*) as the supporting stalk grows out obliquely near the distal end to form a second adhesive cell on the prolongation. The same process may then be repeated, with the second adhesive cell in turn losing its contents and collapsing (FIG. 11, *D, b*) as a third adhesive cell (FIG. 11, *D, c*) is produced. In such successive elongation each new increment is at first continuous throughout. Indeed, young stalks generally (FIG. 12, *C, a*) remain in an unseptate condition until the terminal swelling destined to be delimited as an adhesive knob is nearly full grown.

The glandular knob, if left undisturbed, does not show under ordinary microscopical inspection any recognizable coating of adhesive material on its surface. An eelworm coming in contact with it is nevertheless held securely despite energetic struggles to escape. After these struggles have proceeded for some time, a cushion of colorless glutinous substance often becomes visible between the knob and the integument of the captured animal (FIG. 12, *D, b*; *E, a*; *F, a*). Soon the adhering knob perforates the integument by means of a slender protuberance, which, on reaching the fleshy interior, immediately widens out into a globose infective body that usually continues to expand (FIG. 12, *D*) until it extends entirely or almost entirely across the nematode (FIG. 11, *E–H*; FIG. 12, *E*). The nematode gradually becomes enfeebled from the injury thus sustained, and thereupon is invaded further by assimilative hyphae arising from the infective bodies (FIG. 11, *E–H*; FIG. 12, *E, F*). These assimilative hyphae grow lengthwise through the animal until they occupy it from head to tail (FIG. 12, *G, H*). At first, even in slender eelworms, they are to a considerable degree obscured from view, owing to the globulose degeneration of musculature and organs that accompanies their progress. Later, when the degenerating materials have been largely absorbed, they become more clearly visible. With further reduction of the animal's substance, they show an increasingly vacuolate condition (FIG. 12, *H*) as their protoplasmic contents are withdrawn backward into the external mycelium. Eventually they are completely emptied of living protoplasm, and their membranous envelopes, together with the collapsing integument surrounding them, disappear from sight.

In the withdrawal of protoplasmic contents from the assimilative hyphae into the external mycelium, the passageway provided by the narrow stalk originally supporting the adhesive knob (FIG. 11, *E, b*;

H, a. FIG. 12, *D, b; F, a*) is often supplemented by a passageway provided by a second and noticeably wider hyphal element (FIG. 11, *E, c; H, b.* FIG. 12, *D, c; F, b*) connecting the adhesive knob with the mycelial filament at a point a short distance from the origin of the stalk. This second hyphal connection is often present at an early stage in the invasion of the eelworm, and may possibly be formed to replace the stalk, which, owing to its slenderness, must often incur injury from the struggles of the animal. It may be presumed that localized injury occasions the disappearance of protoplasm within the distal portion of the stalk (FIG. 11, *G, a;* FIG. 12, *H, a*) in the rather frequent instances where the new hyphal element (FIG. 11, *G, b;* FIG. 12, *H, b*) is found arising from a median position in the stalk rather than from the parent mycelial filament. Sometimes the original stalk seems to disappear completely, so that the supplementary hyphal element, recognizable by its greater width, provides the only communication between the adhesive knob and the mycelium (FIG. 11, *E, d.* FIG. 12, *G, a; H, c*). On the other hand, in instances where the captured eelworm is of relatively small size, the adhesive knob may remain attached, at least during the earlier stages of invasion, solely by the stalk on which it was produced (FIG. 11, *F, a;* FIG. 12, *E, a*).

The fungus usually puts forth conidiophores and conidia in moderate abundance both in pure culture and on nematode-infested substratum. Very often, in the beginning, most of the conidiophores present in a stand (FIG. 11, *I, a; J, a; K*) will bear spores singly (FIG. 11, *I, b*) or in twos (FIG. 11, *J, b, c*). At this stage the fungus looks much like a *Dactylella.* Later, however, with the production of additional spores on short branches or spurs, many conidiophores (FIG. 12, *I, a, b; J*) come to bear three (FIG. 12, *I, c–e*) or four (FIG. 12, *I, f–i*) or as many as five conidia in loosely capitate arrangement, thereby acquiring an appearance and habit usually associated with the genus *Dactylaria.* On the whole, capitate development here would seem tardier and less pronounced than in *Dactylaria candida.*

In their handsome fusiform shape the conidia rather closely resemble those of *Dactylella ellipsospora, Dactylella lysipaga,* and *Dactylaria candida,* while differing markedly from the clavate spores of *Dactylella asthenopaga,* as well as from the elongated cylindrical

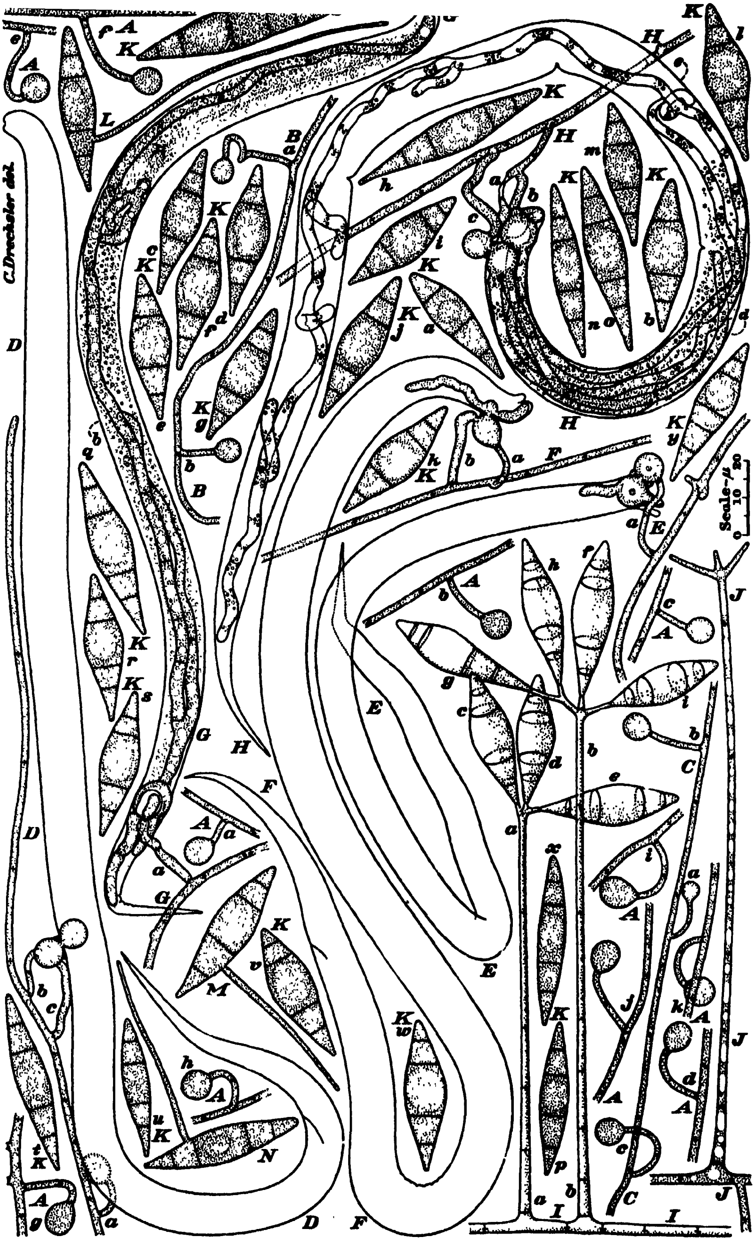

FIG. 12. *Dactylaria haptotyla.*

conidia of *Dactylaria haptospora*. When fully developed they contain three (FIG. 12, *K, a, b*) or four (FIG. 11, *L, a–z;* FIG. 12, *K, c–x*) or occasionally five (FIG. 11, *P;* FIG. 12, *K, y, z*) cross-walls. The quadriseptate condition, with the cross-walls so placed that a largish median cell is delimited between two smaller proximal cells and two smaller distal cells, usually predominates, and thus seems to represent the manner of septation most characteristic of the species. Conidia having such septation give measurements for length ranging from 34 to 55 μ, and measurements for width ranging from 7.4 to 13.3 μ; the computed averages for these dimensions being 43.7 μ and 10.7 μ, respectively. Computed values for average lengths of the five component cells are as follows: basal cell, 9 μ; parabasal cell, 7.3 μ; middle cell, 14.4 μ; penultimate cell, 6.1 μ; apical cell, 6.9 μ. Owing to the greater variability generally evident here in the size and septation of the conidia, the measurements just given must be held less accurately descriptive of the species than are the corresponding measurements, for example, of *Dactylella aphrobrocha.*

Often while it is still borne aloft on the conidiophore, or after it has fallen off, a conidium, whether triseptate (FIG. 11, *M*), quadriseptate (FIG. 11, *N, O;* FIG. 12, *L–N*), or quinqueseptate (FIG. 11, *P*), will extend a slender tapering filamentous outgrowth into the air. These outgrowths, which commonly are 25 to 100 μ long, 1.5 to 2.5 μ wide at the base, and about 1 μ wide near the tip, arise most usually from the parabasal segment. Sometimes a conidium will put forth two such aerial outgrowths (FIG. 11, *Q*). Two detached conidia (FIG. 11, *R, a, b*) lying near together on the surface of a culture often become fused to one another in much the same manner as neighboring mycelial filaments or neighboring assimilative hyphae (FIG. 12, *G, b; H, d, e*).

A term compounded of two words meaning "to fasten," and "knob," respectively, is deemed suitable as specific epithet for the fungus.

Dactylaria haptotyla sp. nov.

Mycelium sparsum; hyphis sterilibus incoloratis, mediocriter septatis, plerumque 1.4–3.8 μ crassis, bullas globosas vel ellipsoideas, 7–10 μ longas, 6–8.5 μ crassas, ex ramulo recto vel curvato, saepius 7–27 μ longo, 1.4–1.9 μ crasso, in 1–3 cellulis consistente, singillatim hic illic emittentibus; his bullis ad vermicu-

los nematoideos inhaerentibus, itaque animalia tenentibus, integumentum eorum perforantibus, tuber mortiferum intrudentibus, hyphas intus evolventibus quae carnem exhauriunt. Hyphae fertiles incoloratae, erectae, saepius 4–11 septatae, 115–325 μ altae, basi 3–4.5 μ crassae, sursum leniter attenuatae, apice circa 1.5 μ crassae, primum saepe simplices et in unum conidium abeuntes, deinde apice saepe parce ramosae denique 2–5 conidia in capitulum laxum ferentes; conidiis hyalinis, vulgo fusoideis, basi truncatis, apice anguste rotundis, plerumque 33–55 μ (saepe circa 43.7 μ) longis, 7.4–13.3 μ (saepe circa 10.7 μ) crassis, 3–5 septatis, saepissime quadriseptatis denique cellula antepaenultima crassiore et longiore quam aliis cellulis.

Vermiculos nematoideos specierum multarum capiens consumensque habitat in foliis caulibusque Poae pratensis putrescentibus prope Beltsville, Maryland.

Mycelium scanty; vegetative hyphae colorless, septate at moderate intervals, mostly 1.4 to 3.8 μ wide, often especially in the presence of nematodes, giving rise here and there on stalks frequently straight or somewhat curved, 7 to 27 μ long, 1.4 to 1.9 μ wide, sometimes unicellular but usually bicellular and occasionally tricellular, to unicellular knobs subspherical or prolate ellipsoidal in shape, 7 to 10 μ long and 6 to 8.5 μ wide; the knobs holding fast to nematodes, individually perforating the integument of the adhering animal, then intruding a globose infective body from which assimilative hyphae are extended to appropriate the fleshy contents. Conidiophores hyaline, erect, often containing 4 to 11 cross-walls, usually 115 to 325 μ high, 3 to 4.5 μ wide at the base, tapering gradually upward, about 1.5 μ wide at the tip, at first often simple and terminating in a single conidium, later often provided near the apex with a few short branches or spurs, and then bearing 2 to 5 conidia in loose capitate arrangement; conidia colorless, usually spindle-shaped, tapering downward toward the narrow truncate base, somewhat narrowly rounded at the distal end, mostly 33 to 55 μ (average about 43.7 μ) long, 7.4 to 13.3 μ (average about 10.7 μ) wide, containing 3 to 5 cross-walls but most often divided by 4 cross-walls into 5 cells whereof the one in middle position usually exceeds the others in length and width.

Capturing and consuming nematodes of different species it occurs in decaying leaves and stems of *Poa pratensis* near Beltsville, Maryland.

A CANDIDA-LIKE DACTYLARIA WITH EFFICACIOUS ADHESIVE KNOBS AND INDURATED HYPHAE

A nematode-capturing fungus which like *Dactylaria haptotyla* resembles *Dactylaria candida* in its conidial apparatus came to light in several maizemeal-agar plate cultures that after being overgrown by my *Pythium arrhenomanes* had been further planted with small quantities of partly decayed vegetable refuse taken from a garden in Roanoke, Virginia, on October 11, 1946. In these cultures the massive lobulate sporangial complexes that were soon produced everywhere by *P. arrhenomanes* served as a very abundant food supply for stylet-bearing nematodes addicted to feeding on fungus protoplasm after the manner described by Christie and Arndt (6) for *Aphelenchoides parietinus* (Bastian **1865**) Steiner **1932**. Consequently the parasitic nematodes prospered greatly, and after two weeks were present in unwonted numbers. About 15 days after the vegetable detritus had been added the stylet-bearing animals, as well as the saprophilous eelworms intermingled with them, began to suffer noticeable losses from the destruction of many individuals by predacious mycelia that grew from the opaque material into the subjacent agar. Capture of nematodes was effected here exclusively by adhesive knobs of a size then known to me—*Dactylaria haptotyla* not having been discovered at the time—only in *Dactylella ellipsospora, Dactylella asthenopaga,* and *Dactylaria haptospora.* When, as soon happened, conidiophores arose from the predacious mycelia, they bore conidia which, while differing markedly from those of *Dactylella asthenopaga* and *Dactylaria haptospora,* resembled in a general way the conidia of *Dactylella ellipsospora* with respect to outward shape and to septation. Agreement with respect to sporulating habit was lacking, however, for the conidia here, instead of being borne for the most part singly, were held aloft plurally in loose heads, thereby offering much the same appearance as *Dactylaria candida.* To determine more especially whether non-constricting rings, familiar as the efficient predacious organs of *Dactylaria candida,* might be formed under changed conditions, the Roanoke fungus was isolated through removal of conidia from the loose heads to sterile agar; and from the pure cultures thus obtained sizable portions of its mycelium were cut out and placed on Petri plate cultures that had become well infested with nematodes originating from different kinds of decomposing plant materials. Development of non-constricting rings never ensued in these trials. The fungus, under varying conditions, continued to capture nematodes solely by means of adhesive knobs.

The adhesive knobs are borne on stalks most often, perhaps, consisting of two cells (FIG. 13, *A, a–q; B, a; C, a, b; D, a; E, a, b*), but nearly as often consisting of a single cell (FIG. 13, *C, c; D, b–e; F; G, a; H, a; K, a*). In stalks composed of more than one cell the basal segment may include a portion of the parent hypha (FIG. 13, *B, b; I; J*), or may even extend into a branch arising nearby from the parent hypha (FIG. 13, *K, b*). The stalks are commonly from 2 to 2.5 μ wide and from 10 to 20 μ long, though some unicellular examples do not greatly exceed 5 μ in length (FIG. 13, *D, b; G, a*), while among the four-celled specimens (FIG. 13, *L*) occasionally to be found some measure about 50 μ in this dimension. They would seem, therefore, somewhat wider than the stalks in *Dactylaria haptotyla,* and, to extend the comparison, are appreciably narrower and also longer than the stalks of *Dactylella ellipsospora.*

The knobs of the Roanoke hyphomycete, like the similar organs in allied species, show no visible coating of adhesive material as long as they are left undisturbed. Nevertheless, eelworms that come into contact with them are held fast despite prolonged struggles to escape. Some little time—frequently about 15 or 30 minutes—after capture of a nematode has been effected, a cushion of hyaline glutinous material can be seen between the knob and the animal's integument (FIG. 13, *D, d*). Soon the knob puts forth centrally in the region of contact a slender protuberance that penetrates the integument to give rise within to a globose infective body. This body usually expands until it occupies the entire width of the animal (FIG. 13, *D, d; E, c, d; G, b, c; H, b; M; N; O*), which thus is severed internally and as a result becomes incapable of energetic movement. Thereupon the infective body produces assimilative hyphae that continue to grow lengthwise through the fleshy interior (FIG. 13, *N, O*) until the eelworm is permeated from head to tail (FIG. 13, *D, d; E, c, d; G, b, c; H, b; M*). At first the assimilative hyphae are only indistinctly visible, being then obscured because of the globulose degeneration marking their progress through musculature and organs; though in slender nematodes, such as the stylet-bearing parasites found utilized as prey in the original cultures (FIG. 13, *D, E, G, H, M, N, O*), the difficulties of observation are less serious than in stouter

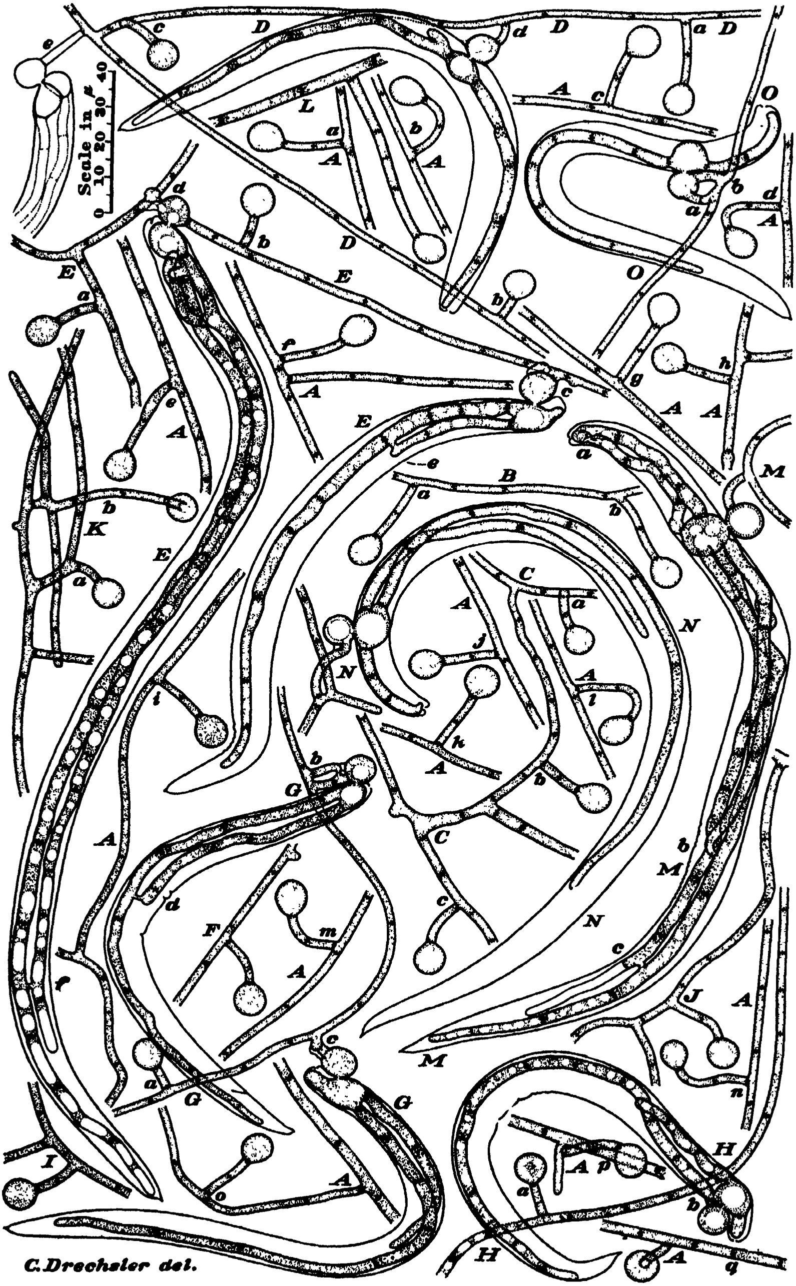

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FIG. 13. *Dactylaria sclerohypha.*

animals. When the degenerating contents have in large part been absorbed by them, the assimilative hyphae, usually numbering from 1 to 3 in slender captives, become more clearly discernible, and reveal cross-walls at moderate intervals as well as scattered instances of vegetative fusion (FIG. 13, *E, e, f; G, d; M, a–c*). The hyphal segments, moreover, now frequently begin to show vacuoles. These vacuoles increase in volume as the animal's contents are further reduced (FIG. 13, *E, c, d; H; M*). Through continued withdrawal of protoplasm backward into the external mycelium, the assimilative hyphae are often completely evacuated of living substance, so that ultimately their empty membranous envelopes, together with the collapsing integument surrounding them and the empty membrane of the associated predacious organ (FIG. 13, *D, e*), gradually vanish from sight.

As in *Dactylaria haptotyla* many adhesive knobs that have been operative in capturing a nematode are found attached to the parent mycelial filament not only by the stalk whereon they were formed (FIG. 13, *O, a*) but also by a supplementary hyphal element (FIG. 13, *O, b*) of later origin. Sometimes a supplementary element intercalated between the knob and the parent hypha may become united laterally with the stalk (FIG. 13, *G, b*), which in its distal portion has been emptied of protoplasm presumably as the result of injury incurred from the struggles of the eelworm. Very frequently, however, where the supplementary element replaces a distal portion of the stalk, it is intercalated between the knob and the proximal living portion of the stalk (FIG. 13, *E, d; G, c; N*). Rather often, again, the stalk is found wholly emptied of protoplasmic material so that the supplementary hyphal element provides the only live communication between the knob and the mycelium (FIG. 13, *E, c*).

On nematode-infested materials, as also in pure culture on maizemeal agar, the fungus usually puts forth a scattered stand of conidiophores. The earliest conidiophores (FIG. 14, *A, a*) are sent up from procumbent mycelial hyphae, but when these have fallen over on the substratum (FIG. 14, *B, a; C; D, a*) after fulfilling their immediate function, they commonly give rise from a segment near the base to a secondary conidiophore (FIG. 14, *B, b; C; D, b*), which in turn may give rise to others of tertiary and

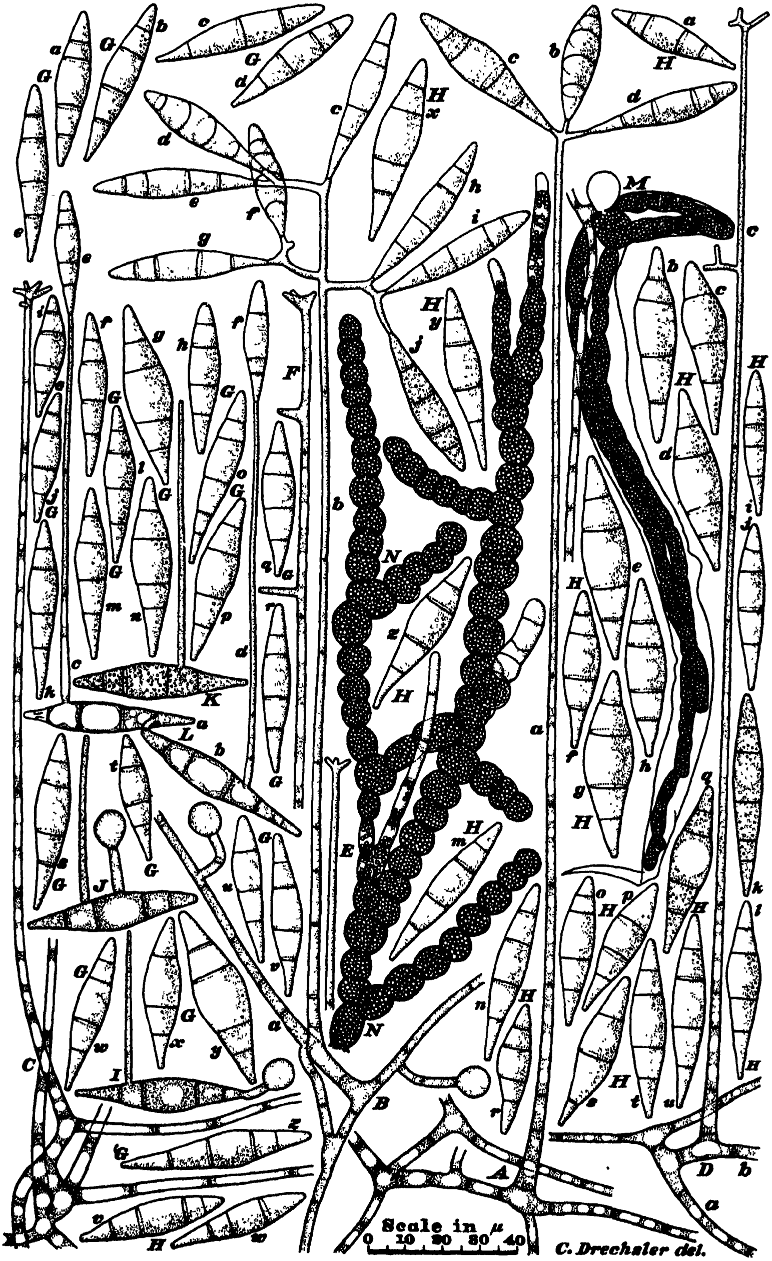

FIG. 14. *Dactylaria sclerohypha.*

higher orders (FIG. 14, *C; D, c*). As a rule the conidiophores are slender in proportion to their length, often seeming hardly sturdy enough to support the conidia, usually 3 to 8 in number (FIG. 14, *A, b–d; B, c–j*), which they hold aloft in loose capitate arrangement 200 to 350 μ above the substratum. In many instances their several sterigmatic spurs are all borne at the tip (FIG. 14, *C, E*), but not infrequently, again, one or more of the spurs are placed some distance below the tip (FIG. 14, *D, c; F*).

The conidia rather closely resemble those of *Dactylaria candida* and *Dactylaria haptotyla* in their dimensions and generally fusiform shape (FIG. 14, *G, a–z; H, a–z*). They are most commonly found divided by four cross-walls, although more than a few have three cross walls (FIG. 14, *G, c, t, u, x; H, c, p, s, w*) and occasional specimens contain five (FIG. 14, *G, o; H, k*). Measurements of quadriseptate specimens have shown a range in length from 32 to 54 μ, and a range in width from 5.9 to 14.3 μ; and have given averages of 44.6 μ and 9.3 μ, respectively, for these two main dimensions. Measurements of the five component cells have yielded averages for lengths as follows: basal cell, 10.8 μ; parabasal cell, 8.4 μ; middle cell, 11.5 μ; penultimate cell, 6.2 μ; and apical cell, 7.7 μ. While in quadriseptate conidia the middle cell would thus seem generally somewhat longer as well as wider than the others, its length, on the whole, does not greatly exceed that of the basal cell, and in individual specimens is found appreciably smaller.

Under warm moist conditions conidia frequently put forth slender aerial filamentous outgrowths while they are still held aloft on the conidiophore. Similar outgrowths are often extended erectly after the spores have fallen on a moist substratum (FIG. 14, *I–K*). In many instances a filamentous outgrowth from a fallen conidium (FIG. 14, *L, a, b*) serves as a conidiophore (FIG. 14, *L, c, d*) in producing at its tip a secondary conidium (FIG. 14, *L, e, f*) usually of smaller dimensions than most conidia of primary origin but having similar shape and septation.

Fallen conidia that happen to lie close together (FIG. 14, *L, a, b*) very frequently become united through vegetative fusion manifestly of the same sort displayed abundantly in mycelial filaments and conidial apparatus throughout the series of clampless predacious hyphomycetes. Another vegetative attribute is shown by fallen conidia in their ready production of stalked adhesive knobs directly from one of their component cells (FIG. 14, *I, J*). Development of like predacious organs on detached conidia was noted earlier in the descriptive account of *Dactylella asthenopaga*. As might well be expected, stalked adhesive knobs are also produced occasionally on conidiophores (FIG. 14, *B, a*) that have fallen over on moist substratum while they were still largely filled with living protoplasm.

After the fungus has been producing conidia in nematode-infested cultures for a period of 10 to 15 days, it often diverts much of its substance toward the formation of chlamydospores or indurated portions of mycelium. Once the change in reproductive tendency has set in, the assimilative hyphae in many instances will not convey their protoplasm to the external mycelium, but instead will retain it within their constituent segments to be thickly interspersed with globulose reserve materials elaborated from the digestible contents of the eelworm (FIG. 14, *M*). In taking on a durable state the hyphal segments here usually become noticeably but not markedly widened. The infective cell, which likewise undergoes induration, reveals generally no further increase in size.

Development of resting bodies through transformation of assimilative hyphae within captured animals has so far never been observed in any other clampless nematode-capturing hyphomycete, nor, for that matter, in the four members of the Zoöpagaceae known to capture eelworms (**10, 11, 14, 17**), nor, again, in the two species of similar biological habit that have been described in the clamp-bearing genus *Nematoctonus* (**18, 20**). Among the various hyphomycetes attacking eelworms after the usual manner of parasites, by means of hyphae arising through germination of affixed or ingested spores, analogous internal development of resting bodies has become familiar to me only in the production of chlamydospores by the ubiquitous *Harposporium anguillulae* Lohde. In the present fungus, besides, scattered filaments of the external mycelium, together with many of their branches, undergo induration to form often rather extensively ramified sclerotioid bodies (FIG. 14, *N*). The hyphal segments here, both in the main filaments and in the branches, frequently become distended to three times their original width, so that the indurated cells are generally of subspherical shape, and in many instances may even measure more in transverse diameter than in length. As in the similar sclerotioid bodies of *Dactylella heterospora,* the more strongly distended cells, which here may form chains over 1 mm. long, are always densely filled with globulose contents, while the cells that have become only slightly distended show correspondingly less internal modification.

A term having reference to the filamentous character of its resting bodies may serve appropriately as specific epithet for the fungus, especially as the resting bodies often occur in a somewhat web-like arrangement.

Dactylaria sclerohypha sp. nov.

Mycelium sparsum; hyphis incoloratis, mediocriter septatis, plerumque 1.5–3.2 μ crassis, hic illic ex ramulo recto vel curvato, 5-50 μ longo, 2–2.5 μ crasso, in 1–4 (saepissime in 1 vel 2) cellulis consistente, bullas tenaces globosas vel ellipsoideas 8.3–10 μ longas, 7.2–8.6 μ crassas, singillatim emittentibus; his bullis ad vermiculos nematoideos inhaerentibus, ita animalia tenentibus, integumentum eorum perforantibus, tuber mortiferum intrudentibus, hyphas plerumque 2–4.5 μ crassas intus evolventibus quae carnem exhauriunt. Hyphae fertiles incoloratae, erectae, saepius 5–10 septatae, 200-350 μ altae, basi 3.3–5 μ crassae, sursum leniter attenuatae, apice 1.4-2 μ crassae, primum saepe simplices et in unum conidium abeuntes, postea apice vulgo aliquid ramosae denique 2-8 conidia in capitulum laxum ferentes; conidiis incoloratis, vulgo fusoideis, apice anguste rotundatis, basi truncatis, plerumque 32–54 μ (saepius circa 44.6 μ) longis, 5.9-14.3 μ (saepius circa 9.3 μ) crassis, 3–5 septatis, saepissime quadriseptatis denique cellula antepenultima eorum plerumque crassiore et longiore quam aliis cellulis, post disjunctionem bullam tenacem vel hypham fertilem interdum emittentibus; hyphis fertilibus germinationis simplicibus saepius circa 100 μ altis, basi 2–2.5 μ crassis, sursum leniter attenuatis, apice circa 1.2 μ crassis, saepius biseptatis vel triseptatis, unum conidium ferentibus; conidiis ordinis secundi fusiformibus plerumque triseptatis vel quadriseptatis, vulgo circa 32 μ longis et 6.5–7 μ crassis. Corpora perdurantia intra animalis atque in materia circumdanti orta, filiformia, vulgo ramosa, paene incolorata vel flavidula, protoplasmatis valde guttulosi repleta, saepe 0.2–1 mm. longa, in cellulis plerumque 8–15 μ longis et 3–13 μ crassis consistentia.

Vermiculos nematoideos multarum specierum capiens consumensque habitat in materiis plantarum putrescentibus prope Roanoke, Virginia.

Mycelium scanty; vegetative hyphae colorless, septate at moderate intervals, mostly 1.5 to 3.2 μ wide, often especially in the presence of nematodes giving rise here and there on straight or curved stalks, 5 to 50 μ long and 2 to 2.5 μ wide, usually uniseptate or biseptate but sometimes triseptate or quadriseptate, to solitary adhesive knobs, globose or ellipsoidal in shape, commonly 8.3 to 10 μ long and 7.2 to 8.6 μ wide; these knobs holding fast to nematodes, thus capturing the animals, then perforating the integument of each captive and intruding a globose infective body from which assimilative hyphae, mostly 2 to 4.5 μ wide, are extended lengthwise to appropriate the fleshy contents. Conidiophores colorless, erect, often containing 5 to 10 cross-walls, frequently 200 to 350 μ high, 3.3 to 5 μ wide at the base, gradually tapering upward to a width of 1.4 to 2 μ at the tip, at first often simple and terminating in a single conidium but later often provided distally with short branches or spurs and then bearing 2 to 8 conidia in loose capitate arrangement; conidia colorless, commonly spindle-shaped, narrowly rounded at the tip, truncate at the base, mostly 32 to 54 μ (average about 44.6 μ) long, 5.9 to 14.3 μ (average about 9.3 μ) wide, containing from 3 to 5 cross-walls but most often divided by 4 septa into 5 cells whereof the middle one is widest and usually longest, after being abjointed sometimes putting forth a stalked adhesive knob or a conidiophore; conidiophores of germinative origin usually simple, often biseptate or triseptate, about 100 μ high, 2 to 2.5 μ wide at the base, tapering gradually upward to a width of 1.2 μ at the tip, whereon is borne a single secondary conidium; secondary conidia spindle-shaped, mostly triseptate or quadriseptate, commonly about 32 μ long and 6.5 to 7 μ wide. Resting bodies formed within the animal's integument and also externally, filamentous, frequently branched, nearly colorless or faintly yellowish, often 0.2 to 1 mm. long, composed of cells mostly 8 to 15 μ long and 3 to 13 μ wide that are filled with densely globuliferous protoplasm.

Capturing and consuming nematodes of different species it occurs in decaying plant materials near Roanoke, Virginia.

A CLAMPLESS HYPHOMCETE CAPTURING NEMATODES IN THICK NON-CONSTRICTING RINGS

The several maizemeal-agar plate cultures which after being planted with leaf mold from woods near Greensboro, North Carolina, permitted abundant development of *Dactylella aphrobrocha,* were occupied rather extensively also by a number of other clampless hyphomycetes of like predacious habit. Among these allied fungi was a species that produced three-celled non-constricting rings (FIG. 3, *S; T; U, a; V*) and globose or ellipsoidal unicellular knobs (FIG. 3, *U, b*) on delicate, mostly bicellular stalks often about 25 μ in length. While the development of these two types of predacious organs on the same mycelium offered general parallelism with development in *Dactylella lysipaga, Dactylella leptospora,* and *Dactylaria candida,* the rings here could immediately be recognized as being larger and more massive than those of the three species mentioned. They measured from 20 to 26 μ in outside diameter and thus with respect to this dimension exceeded the rings of the three similar species by approximately 5 μ. In respect to thickness of the three cells composing them they ranged from 3 to 5 μ, their more usual transverse measurement of approximately 4 μ exceeding the corresponding measurement in the three known similar forms by about 1 μ. On the whole they would appear, indeed, very nearly equal in size and volume to the constricting rings of *Dactylella stenobrocha.* This approximate equality provides an exception to the marked dimensional disparity generally observable among annulated members of the predacious series, between the rather frail non-constricting rings and the conspicuously sturdy constricting rings.

For the most part the rings operated like those of *Dactylella lysipaga, Dactylella leptospora,* and *Dactylaria candida.* In some instances the slender supporting stalk withstood the frantic struggles of the ensnared nematode, though often incurring injury distally (FIG. 3, *W, a*) that needed to be repaired through intercalation of a new hyphal connection (FIG. 3, *W, b*). In other instances the stalk broke, permitting the animals, now tightly encircled by the ring (FIG. 3, *X, a*) to continue moving about for a few hours longer. In either event the ring would soon put forth from its inner surface a narrow protuberance which after penetrating the animal's integument gave rise within the fleshy interior to a globose infective body. Owing apparently to the greater volume of protoplasm contained in the ring the infective body here continued to enlarge, as a rule, until it occupied the whole width of the eelworm, whereas in the three species with smaller rings it commonly extends only partly across the animal. The virtually complete severance of musculature and organs promptly resulted in disablement of the eelworm, thereby making possible early extension of assimilative hyphae from the infective body throughout the fleshy interior. Where a nematode, after breaking the stalk, moved some distance from the predacious mycelium before succumbing to infection, its substance was utilized by the fungus to put forth from the rings one or more external hyphae that grew out to establish a new tract of predacious mycelium (fig. 3, *X, b*).

The knobs were never observed taking any part in the destruction of eelworms. It may be presumed that in this species as also in *Dactylella lysipaga, Dactylaria haptospora,* and *Dactylaria candida,* these relatively small organs are designed to function under conditions somewhat different from those suitable for the operation of non-constricting rings—under conditions apparently not often occurring in agar plate cultures of the sort employed by me. Although the mycelium bearing the two kinds of predacious organs was kept under observation until it degenerated from age, it has not so far been found giving rise to conidiophores and conidia, nor has it been seen associated with any other type of reproductive apparatus. The fungus would appear distinct from *Anulosporium nematogenum* Sherbakoff (**30**) in view of the larger dimensions of its rings. Its further characterization is deferred until the discovery of a conidial stage may permit reference to the proper genus in the predacious series of hyphomycetes.

GONATOBOTRYS SIMPLEX AND ITS RELATIONSHIP TO THE PREDACIOUS HYPHOMYCETES

In the classic "Pracht-Flora" wherein Corda (**9**) more than a century ago published the original account of his *Arthrobotrys superba* he likewise presented the original description of his *Gonatobotrys simplex.* The two fungi were set forth as being similar in their development of conidiophores with swollen nodes, in the spiral arrangement of warty sporiferous protuberances on the nodes, and in their production of oblong spores in successive capitate clusters. Owing, however, to difference in cellular make-up of their spores each was presented, in conformity with the requirements of taxonomic practice, as the type of a separate new mucedinous genus; unseptate conidia being thereby specified for the genus *Gonatobotrys,* and uniseptate conidia for *Arthrobotrys.* De Bary made the two genera more familiar by citing them together, in both editions of his well known manual on fungi (**1**: 46, 47; **2**: 50), as exemplifying successive development of conidial clusters on a repeatedly prolonged conidiophore. The unseptate conidia specified for it gave occasion for assignment to *Gonatobotrys* of such diverse species as *G. microspora* Rivolta (**28**) and *G. pallidula* Bresadola (**5**): the former, with its continuous mycelium, having subsequently been recognized as a phycomycete and enrolled among the Mucorales under the binomial *Cunninghamella microspora* (Riv.) Matruchot (**26**: 56; **31**: 168); whereas the latter, with clamp-connections in its mycelial filaments, has been transferred to the resupinate basidiomycetes as *Peniophora pallidula* (Bres.) Bres. apud Bourdot et Galzan (**4**, **29**). Neither the unhappy application of *Gonatobotrys*, nor the persistent confusion of *Arthrobotrys oligospora* with *Trichothecium roseum* Link to which some discussion was given earlier (**12**: 469–472), can be held to have upset the parallelism pointed out by Corda, as far as this parallelism concerns forms wherein the sporiferous and mycelial hyphae are divided by ordinary cross-walls. The possibility is not to be dismissed that among septate clampless hyphomycetes such parallelism might in some instances, if not generally, derive from close kinship, and thus might be associated with biological similarities. For this reason, after *A. superba*, together not only with *A. oligospora* and other congeners but also with many related hyphomycetes producing multiseptate spores of various sizes and shapes, was found subsisting habitually through capture of nematodes, cultures infested with eelworms have been observed closely for the development of predacious fungi referable to *Gonatobotrys*. In the course of more than a decade, however, no clampless hyphomycete bearing unicellular conidia in successive or in solitary clusters has ever been found preying on eelworms in infested agar cultures planted with decaying vegetable materials of different kinds. Therefore, when some years ago a true *Gonatobotrys* appeared abundantly on tomato (*Lycopersicon esculentum* Mill.) and muskmelon (*Cucumis melo* L.) leaves in the laboratory, opportunity was taken to try it out on nematodes, and also to grow it in pure culture for comparison with familiar members of the predacious series of clampless hyphomycetes.

The muskmelon leaves here in question were gathered in a field near Baltimore, Maryland, on July 13, 1942, and bore large dry lesions due evidently to *Alternaria cucumerina* (Ell. & Ev.) J. A.

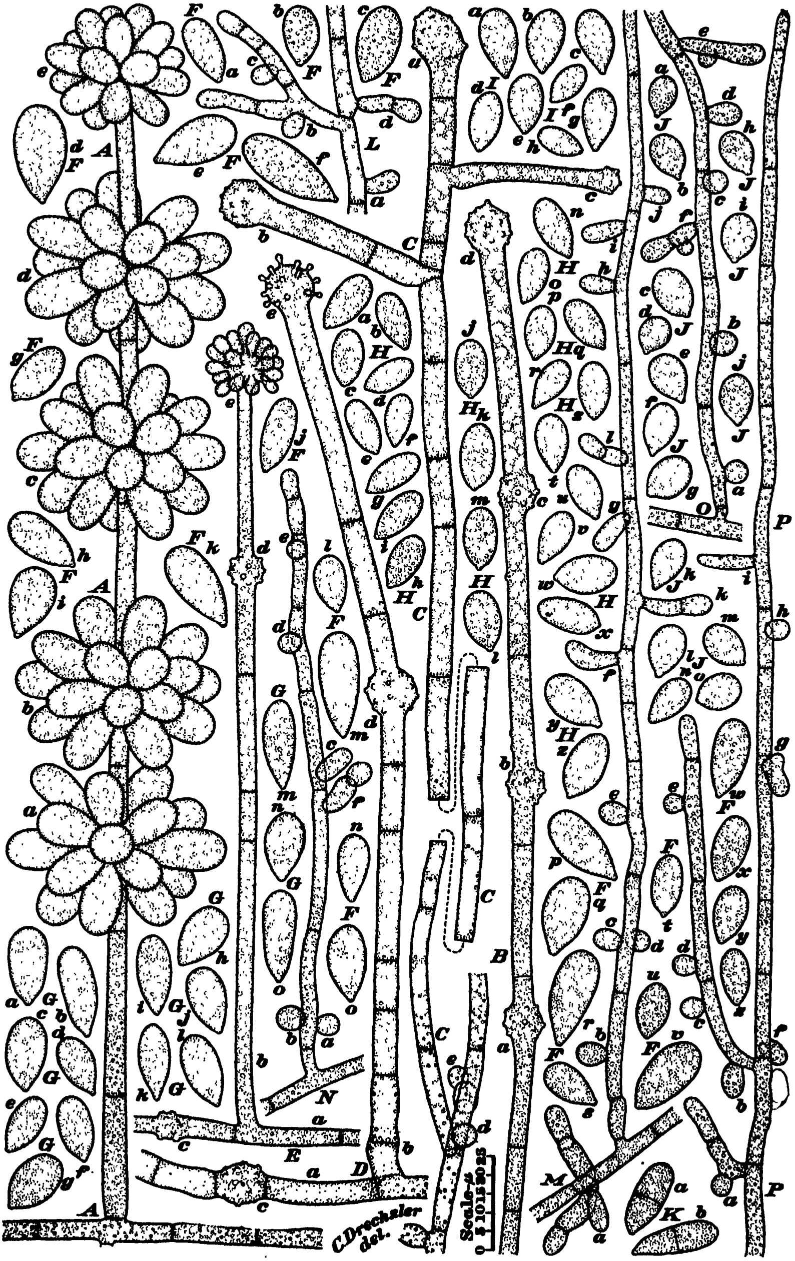

FIG. 15. *Gonatobotrys simplex.*

Elliott. Rather similar lesions attributable to the early-blight fungus, *Alternaria Solani* (Ell. & Martin) Sor., were present on the tomato leaves when these were collected in a field near Beltsville, Maryland, on July 25, 1942. Soon after their removal from the plants the leaves were arranged in a moist chamber and stored at a temperature of 18° C. On examining them 10 days later they were found covered extensively with several different molds. The *Gonatobotrys* appeared as a white compact layer covering irregular patches mostly 1 to 5 square centimeters in area. It offered to the naked eye a delicately granular appearance; the individual granules becoming recognizable under the microscope as conidial clusters (FIG. 15, *A*, *a–e*) borne at intervals of 20 to 100 μ on erect or drooping conidiophores mostly 4.5 to 7 μ wide. When small tracts of the sporulating layer were mounted in water under a cover-glass, virtually all mature conidia became abjointed from the conidiophores, exposing to view the denuded nodes, usually 8 to 12 μ wide, and beset with truncated wart-like protuberances (FIG. 15, *B*, *a–d*). In pure culture on maizemeal agar the conidiophores frequently bore at some little distance below the expanded sporiferous tip (FIG. 15, *C*, *a*) one or more lateral branches each of which similarly terminated in a fertile tip often perceptibly swollen (FIG. 15, *C*, *b*, *c*). Such branching, as a rule, was associated with less abundant development of spore clusters on the repeatedly prolonged axial hypha. Older conidiophores, both in pure culture (FIG. 15, *D*, *a*) and on the softened leaves (FIG. 15, *E*, *a*), often declined to the substratum, and would then send up an erect branch (FIG. 15, *D*, *b*; *E*, *b*) in a position usually a little removed from any of its nodes (FIG. 15, *D*, *c*; *E*, *c*). Through the production of spore clusters at successive nodes (FIG. 15, *D*, *d*, *e*; *E*, *d*, *e*) these branches served as secondary conidiophores.

The development of the several conidia in a cluster appears here more strictly simultaneous than in any of the species of *Arthrobotrys* and *Dactylaria* that are known to capture eelworms. At the earliest stage in the formation of a cluster the distended tip of the conidiophore is found bearing from twenty to thirty protuberances, all equally minute (FIG. 15, *D*, *e*); the appearance then given recalling young sporophores of *Cunninghamella echinulata* (Matr.) Thaxter. Growth of the protuberances proceeds steadily, each of them keeping pace accurately with the enlargement of its fellows (FIG. 15, *A, e; E, e*), so that all reach definitive size and are ready to be abjointed as conidia at the same time (FIG. 15, *A, a–d*). Conidia produced on tomato and muskmelon leaves are generally of an elongated obovoid shape though noticeably apiculate at the basal end (FIG. 15, *F, a–z; G, a–o; H, a–z; I, a–h*). They measure commonly from 12 to 29 μ in length and from 7 to 13.5 μ in greatest width. Conidia produced in pure culture on maizemeal agar are of more broadly obovoid conformation and of smaller dimensions, their length varying ordinarily from 11 to 15 μ, and their greatest width from 7 to 10 μ (FIG. 15, *J, a–o*). The unseptate condition is always characteristic of the conidia, regardless of the substratum on which they are formed. However, in some mounts of material produced on tomato leaves a few uniseptate specimens (FIG. 15, *K, a, b*) were found among many thousands wherein a cross-wall was lacking. These closely resembled the conidia of *Arthrobotrys superba* and may, perhaps, have been present as an incidental admixture of that species.

In pure culture on maizemeal agar the fungus grows more slowly than the larger number of clampless hyphomycetes that are known to subsist by capturing eelworms. Nevertheless in 10 to 15 days it usually gives rise to aerial hyphae and conidiophores in easily recognizable quantity. To the naked eye the aerial weft offers a fairly pronounced orange coloration decidedly deeper in shade than the pink coloration characteristic of the conidial apparatus formed so abundantly in pure cultures of *Dactylaria polycephala*. When sizable portions of agar permeated with young mycelium of the fungus were transferred to agar plate cultures infested with various eelworms, amoebae, and testaceous rhizopods, no predacious or parasitic characteristics came to light. From such trials, however, it might be premature to deny all possibility of the fungus subsisting on animals, since in pure culture its mycelial hyphae are regularly found bearing globose or digitate lateral branches that consist commonly of one (FIG. 15, *L, a–c; M, a–j; N, a–e; O, a–e; P, a–i*) or two (FIG. 15, *L, d; M, k, l; N, f; O, f*) cells, and thereby in some degree resemble the adhesive outgrowths of *Dactylella cionopaga*. Though these curious lateral branches have shown no efficacy for the destruction of the few types of animals present in my cultures, the large variety of minute animals capable of multiplying on the lower leaves of vegetable crop plants, especially during prolonged periods of wet weather, might well offer scope for many biological relationships not hitherto revealed to sight.

As my fungus when developing on leaves in the dark at a temperature of 18° C. agrees rather well with Corda's description of *Gonatobotrys simplex,* it is assigned to that ancient species,—the assignment appearing further justified through observations by Coemans (**7**), Harz (**23**), and Matruchot (**25**) which show that wide variability with respect to coloration and branching is usual here. On grounds of priority Corda's binomial seems preferable to some others with which it has been held synonymous; for *Gonatobotrys* clearly antedates the genus *Oedocephalum* Preuss (**27**: 131) as well as the genus *Desmotrichum* Léveillé (**24**), while similarly the species *G. simplex* antedates *G. flava* Bon. (**3**: 105), *G. ramosa* Riess ex Fresenius (**21**), and *Oedocephalum roseum* Cooke (**8**). The relationship of the fungus to *Arthrobotrys* and to the series of clampless predacious hyphomycetes remains problematical. Although the general parallelism in manner of conidial development argues for kinship, the protoplasm in the conidiophore seems of markedly different texture from that in most mucedinous forms known to capture eelworms. Woronin bodies are either absent here or far less readily discernible than in the familiar species of *Arthrobotrys.* The rather distinctive odor given off by maizemeal-agar cultures of all known nematode-capturing species of *Arthrobotrys* has never been detected in any cultures of the *Gonatobotrys.*

Since Corda set forth *Gonatobotrys simplex* as growing parsitically on *Helmisporium tenuissimum* Nees, it was deemed appropriate to try out my fungus on two dematiaceous forms—*Alternaria Solani* and *Alternaria tenuis* Nees—that developed abundantly nearby on the same tomato leaves. Accordingly sizable portions of agar well permeated with young mycelium were taken from pure cultures of the *Gonatobotrys* and placed on maizemeal-agar plate cultures of the two species of *Alternaria.* In these trials the *Gonatobotrys* showed no capacity for parasitizing the two dematiaceous forms.

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EXPLANATION OF FIGURES

FIG. 1. *Dactylella stenobrocha* as found developing in nematode-infested agar plate cultures; drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* Portion of mycelial hypha bearing an open predacious ring in its original position. *B–E,* Portions of hypha, each bearing an open predacious ring that is turned sideways, thereby showing advantageously its three arcuate cells and the two cells making up the stalk. *F,* Longer portion of hypha bearing two open predacious rings, *a* and *b,* that are turned sideways, thereby revealing their cellular make-up advantageously. *G,* Portion of hypha bearing a predacious ring that has closed emptily without capturing an eelworm; the ring being turned sideways shows advantageously the shape and vacuolate condition of the contracted arcuate cells. *H,* Portion of hypha with a ring that has contracted in capturing a specimen of *Plectus* sp.; two stout protuberances, one extending from the closed ring forward within the animal, and the other extending from the closed ring backward within the animal, represent assimilative hyphae in an early stage of development. *I,* Portion of hypha with a ring that has contracted in capturing a specimen of *Plectus* sp.; the two assimilative hyphae, one extending forward and the other backward within the animal, have attained their definitive length, with each showing some swelling of its terminal segment *J,* Portion of hypha bearing a closed ring that holds a captured specimen of *Plectus* sp.; the two long assimilative hyphae extending to the animal's head and tail, respectively, each show some enlargement of the two terminal segments and the pronounced vacuolization of all segments that comes with advanced exhaustion of fleshy substance. *K,* Portion of prostrate hypha, *a,* with two erect conidiophores, *b* and *c,* whereon are borne the conidia *d* and *e,* respectively; each conidiophore being shown, from want of space, in three parts whose proper connection is indicated by broken lines. *L,* Portion of prostrate hypha, *a,* with a fallen conidiophore, *b,* that has given rise to an

erect secondary conidiophore, *c*, which bears at its tip the well developed conidium *d;* the conidiophore *c* being shown, from lack of space, in three parts whose proper connection is indicated by broken lines. *M*, Short basal portion of a fallen conidiophore, *a*, from which has been sent up the erect secondary conidiophore *b*, bearing terminally a conidium, *c;* the conidiophore *b* being shown, from lack of space, in three parts whose proper connection is indicated by broken lines. *N*, Mature detached conidia showing usual variations in size and shape, together with variations in number and position of septa as follows: *a–c*, single septum near the proximal end; *d–o*, two septa near the proximal end; *p–s*, two septa symmetrically placed, one near the proximal end, the other near the distal end; *t–z*, three septa, two near the proximal end, one near the distal end. *O*, Two conidia, *a* and *b*, each with three septa placed near the proximal end.

FIG. 2. *Dactylella stenobrocha* as found developing in nematode-infested agar plate cultures; drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, Portion of mycelial hypha bearing two predacious rings, *a* and *b*, in their original positions. *B–E*, Portions of hypha, each bearing a predacious ring that is turned sideways, thereby showing its cellular make-up advantageously. *F*, Short portion of hypha with a closed predacious ring nearly in its original position. *G*, Portion of hypha with a predacious ring that has closed in capturing a specimen of *Plectus* sp.; no penetration into the animal has yet taken place. *H*, Portion of hypha whereon is borne a predacious ring, *a*, that closed in capturing a specimen of *Plectus* sp. and then invaded the animal with three assimilative hyphae, two of which are rather markedly distended in their two terminal segments; nearby is attached a second predacious ring, *b*, that has closed emptily without capturing any eelworm. *I*, Portion of hypha with a predacious ring that has closed in capturing a specimen of *Plectus* sp., and has extended an assimilative hypha forward, and another one backward through the animal's body; the last two segments of the hypha directed forward each showing marked enlargement. *J*, Portion of hypha with a predacious ring that has closed in capturing a specimen of *Plectus* sp., and has given rise to assimilative hyphae extending forward and backward through the animal's body; *a*, anastomosis of two assimilative hyphae. *K*, *L*, Portions of prostrate hypha, each with an erect conidiophore, *a*, near the tip of which is shown a conidium, *b*, that was abjointed from it; the conidiophore *a* in each instance is shown, from lack of space, in three parts whose proper connection is indicated by broken lines. *M*, Mature detached conidia, showing usual variations in size and shape, together with variations in number and position of septa as follows: *a*, single septum near basal end; *b–m*, two septa near the basal end; *n*, *o*, two septa symmetrically placed, one near the proximal end, the other near the distal end; *p–z*, three septa, two of them near the proximal end, the other near the distal end. *N*, Conidium with three septa, two of them near the proximal end, the other one near the distal end. *O*, Detached triseptate conidium that has sent up an aerial hypha from its small apical segment. *P*, Detached biseptate conidium that has sent up an aerial hypha from its basal segment. *Q*, Abjointed triseptate conidium that has sent up two aerial hyphae, one from its small parabasal segment, the other from its small apical segment. *R*, Detached conidium that has put forth a germ tube from a position next to its base.

FIG. 3. Drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A, B,* Portions of hyphae, each with a denuded conidiophore of *Dactylella stenobrocha* as found in nematode-infested agar plate cultures; each conidiophore being shown, from a lack of space, in three parts whose proper connection is indicated by broken lines. *C–H, Dactylella stenobrocha* as found developing in pure culture on maizemeal agar: *C–E,* Portions of hyphae, each with a conidiophore from whose tip a single conidium has been abjointed. *F,* Uniseptate conidia, *a–o,* showing usual variations in size and shape. *G,* Biseptate conidia showing usual variations in size and shape, some of them (*a–h*) having both cross-walls placed near the proximal end, and others (*i–p*) having one cross-wall near each end. *H,* Triseptate conidium. *I,* Detached conidium of *Dactylella aphrobrocha* produced in a nematode-infested agar plate culture that had been planted with deciduous leaf mold gathered near Fairfax, Virginia, in November, 1942. *J–R, Dactylella ctenopaga* as found developing in nematode-infested agar plate cultures that had been planted with deciduous leaf mold gathered near Butternut, Wisconsin, in September, 1938: *J,* Portion of hypha bearing a simple three-celled predacious outgrowth, *a,* and a branched seven-celled predacious outgrowth, *b.* *K,* Portion of hypha with three predacious outgrowths, *a–c,* to which is affixed a small stylet-bearing nematode; a globose infective body intruded from the two-celled outgrowth *c* has given rise within the animal to a growing assimilative hypha. *L,* Portion of hypha with four predacious outgrowths, *a–d,* of which three (*b–d*) have jointly captured a small nematode (probably referable to *Plectus* sp.) and have each intruded a bulbous infective body into the captive; the several assimilative hyphae extended from the infective bodies have become joined through vegetative fusion. *M, N,* Detached triseptate conidia. *O–Q,* Detached quadriseptate conidia. *R,* Quadriseptate conidium germinating by two polar germ tubes. *S–X,* Unnamed hyphomycete found capturing nematodes in agar plate cultures that had been planted with leaf mold gathered near Greensboro, North Carolina, in December, 1947. *S, T,* Portions of mycelial hyphae, each bearing a non-constricting ring. *U,* Portion of mycelium bearing a non-constricting ring, *a,* and a globose adhesive cell, *b.* *V,* Portion of hypha with a non-constricting ring. *W,* Portion of hypha with a non-constricting ring that after having operated in capturing a nematode (possibly referable to *Wilsonema* sp.) has intruded into the animal a globose infective body from which four assimilative hyphae are being extended lengthwise through the fleshy interior; the narrow stalk, *a,* originally supporting the ring has been evacuated distally, and a new connection, *b,* has been formed. *X,* Small eelworm that after being captured in a non-constricting ring, *a,* tore loose the encircling organ but was nevertheless later killed through intrusion of a globose infective body from which it was then invaded throughout by assimilative hyphae; a mycelial hypha, *b,* grew out externally from the ring.

FIG. 4. *Dactylella aphrobrocha* as found developing in nematode-infested agar plate cultures; drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* Portion of hypha bearing a large predacious ring in its original position. *B,* Portion of hypha bearing an open

predacious ring, *a*, in its original position, and another such ring, *b*, displaced sideways a little; the stalk supporting ring *b* is unusual in length, and in being composed of three cells. *C*, Portion of hypha with two open predacious rings, *a* and *b*, both being turned sideways out of their original positions. *D*, Portion of hypha bearing an open predacious ring of relatively small size. *E*, Portion of hypha with a rather large open predacious ring that is turned sideways a little. *F–H*, Portions of hypha, each bearing an open predacious ring that is turned flatwise, thereby advantageously showing its cellular make-up. *I*, Portion of hypha with two open predacious rings, *a* and *b*, both being turned flatwise. *J*, Portion of hypha with a closed predacious ring that is turned flatwise. *K*, Portion of hypha with a predacious ring that has closed in capturing an eelworm (possibly referable to *Plectus parvus* Bastian) and has given rise to assimilative hyphae within the animal. *L*, Portion of hypha with a predacious ring that after capturing an eelworm has extended several assimilative hyphae lengthwise through its body; these hyphae have very nearly exhausted the animal's fleshy substance, and are becoming vacuolate from transfer of protoplasm to the external mycelium. *M, N*, Portions of prostrate hyphae, each bearing an erect conidiophore, *a*, near the tip of which is shown a conidium, *b*, that was abjointed from it; the conidiophore *a* being shown in each instance, from lack of space, in three parts whose proper connection is indicated by broken lines. *O*, Random assortment of detached mature conidia, showing usual variations in size and shape, together with variations with respect to number and position of septa as follows: *a–c*, three septa present, two of them placed near the proximal end, and one near the tip; *d*, three septa present, one of them placed near the proximal end, and two near the tip; *e–z*, four septa present, in symmetrical arrangement, two being placed near each end. *P, Q*, Symmetrically quadriseptate conidia, each of which has extended an aerial hypha from its rather small parabasal cell. *R*, Symmetrically quadriseptate conidium that has put forth two aerial hyphae, one from the penultimate segment, the other from the parabasal segment. *S, T*, Symmetrically quadriseptate conidia, each germinating by putting forth a germ tube from its basal and its apical cell.

FIG. 5. *Dactylella aphrobrocha* as found developing in nematode-infested agar plate cultures; drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, Portion of mycelial hypha bearing a large open predacious ring in its original position. *B*, Portion of hypha with an open predacious ring that has been turned sideways to a considerable degree. *C–H*, Portions of hyphae, each with an open predacious ring that is turned flatwise, thereby showing advantageously usual variations in size and shape of the arcuate cells, the stalk cells, and the aperture. *I*, Several successive stages in the formation of a predacious ring; *a*, simple branch of circinate curvature, with two cross-walls delimiting the two cells of the stalk; *b*, same branch 1½ hours later, showing its tip directed toward the tip of a secondary spur that is growing out from the distal segment of the stalk; *c*, same branch 30 minutes later than *b*, showing its tip in contact with the tip of the spur, and presence of a third cross-wall, which delimits the proximal arcuate cell; *d*, same branch 30 minutes later than *c*, showing fusion of tip and spur, and continued homogeneous appearance of protoplasmic contents throughout the ring and its stalk; *e*, same branch 17 hours later than *d*,

showing completed ring in functional condition, each of the arcuate cells containing an irregular elongated lacuna of obscurely globulose or foamy composition. *J*, Portion of hypha with a rather small predacious ring in lateral view; the stalk here being exceptional in consisting of three cells. *K*, Portion of hypha with a predacious ring which after capturing a small eelworm (possibly referable to *Plectus parvus*) has given rise to young assimilative hyphae within the animal. *L*, Portion of hypha with a predacious ring which after capturing a small eelworm has given rise to assimilative hyphae extending lengthwise through the animal. *M*, Portion of mycelium with a predacious ring which after capturing an eelworm of larger size has given rise to assimilative hyphae extending lengthwise through the animal; the stalk *a* originally supporting the ring having suffered injury, a new connection, *b*, has been formed between the mycelial hypha and one of the swollen cells. *N*, Portion of hypha with a predacious ring which after capturing an eelworm gave rise to assimilative hyphae extending lengthwise through its body; the original stalk, *a*, and two of the swollen cells having incurred destructive injury, a new hyphal connection, *b*, has been formed between the parent mycelial filament and the irregular proximal infective cell of the assimilative hyphal system. *O*, Portion of prostrate hypha bearing an erect conidiophore, *a*, which on its axial tip and on the tip of a branch, *b*, bore the conidia *c* and *d*, respectively; the conidiophore, from lack of space, being shown in three parts whose proper connections are indicated by broken lines. *P*, Random assortment of conidia, showing usual variations in size and shape, together with variations in number and position of septa as follows: *a–c*, three septa present, whereof two are placed near the proximal end, and one near the distal end; *d–w*, four septa present in symmetrical arrangement, two being placed near each end.

FIG. 6. *Dactylella cionopaga* drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, *B*, Portions of hypha, each bearing a unicellular dome-shaped adhesive outgrowth. *C*, Portion of prostrate hypha bearing a unicellular columnar adhesive outgrowth, *a*, two bicellular adhesive outgrowths, *b* and *c*, and a five-celled adhesive arch, *d*. *D*, Portion of prostrate hypha bearing a four celled columnar adhesive outgrowth, *a*, together with a three-celled adhesive arch, *b*, and a six-celled adhesive arch, *c*; the adhesive arch *b* is surmounted by a two-celled adhesive process. *E*, Portion of prostrate hypha with a low adhesive protuberance, *a*, arising from one of its segments; on the hypha are borne further a two-celled adhesive outgrowth, *b*, three four-celled columnar adhesive outgrowths, *c–e*, and an eight-celled network of two meshes, *f*. *F*, Portion of prostrate hypha with a six-celled adhesive outgrowth. *G*, Portion of hypha with a three-celled adhesive outgrowth which after capturing a slender eelworm (possibly referable to *Plectus* sp.) has intruded three bulbous excrescences into the animal; the excrescence intruded from the basal segment having given rise to a short, growing assimilative hypha. *H*, Portion of prostrate hypha with a three-celled adhesive process that after capturing a slender eelworm has intruded into the animal a bulbous excrescence from which a short, growing assimilative hypha has been extended. *I*, Portion of prostrate hypha bearing a two-celled columnar adhesive outgrowth that after capturing a slender eelworm has intruded into the animal a bulbous excrescence from which one

assimilative hypha has been extended forward and another backward through the fleshy body. *J*, Portion of prostrate hypha with two bicellular adhesive outgrowths, *a* and *b*, each of which has intruded a bulbous excrescence into a slender eelworm that they had captured jointly; assimilative hyphae have begun growing out from each of the intruded bodies. *K*, Portion of prostrate hypha with a unicellular adhesive outgrowth, *a*, and a two-celled adhesive outgrowth, *b*, which have each intruded a bulbous excrescence into a slender eelworm captured jointly by them; assimilative hyphae extended from the two bulbous bodies have become united by an anastomosing connection, *c*. *L–N*, Portions of prostrate hyphae, each bearing an erect conidiophore from which a conidium has been abjointed. *O*, Mature detached conidia, showing usual variations in size and shape, together with variations with respect to septation as follows: *a*, two cross-walls present; *b–f*, three cross-walls present; *g–n*, four cross-walls present; *o*, *p*, five cross-walls present; *q*, six cross-walls present. *P*, Fallen conidium that has put forth a two-celled adhesive outgrowth, *a*, from its distal end. *Q*, *R*, Fallen conidia, each of which has put forth one two-celled adhesive outgrowth, *a*, from its proximal cell, and another, *b*, from its distal cell. *S*, Fallen triseptate conidium which has given rise directly to three sessile adhesive outgrowths, *a–c*, besides putting forth at its proximal end a stout hypha that bears on the side a unicellular adhesive process, *d*, and at the tip has a two-celled adhesive part, *e*. *T*, Fallen conidium which at one end has put forth directly a sessile adhesive outgrowth, *a*, shown as viewed endwise, and at the other end has produced a stout hypha bearing two bicellular adhesive outgrowths, *b* and *c*.

FIG. 7. *Dactylella cionopaga*, drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, Portion of mycelial hypha bearing a two-celled columnar adhesive outgrowth, *a*, and a three-celled columnar adhesive outgrowth, *b*. *B*, Portion of mycelial hypha whereon are borne a unicellular adhesive outgrowth, *a*, a two-celled columnar adhesive outgrowth, *b*, and a six-celled adhesive arch, *c*. *C*, Portion of prostrate hypha with a five-celled adhesive arch formed by distal union of two outgrowths. *D*, Portion of prostrate hypha bearing in addition to an inactive two-celled adhesive outgrowth, *a*, two other adhesive outgrowths, *b* and *c*, that have been operative in capturing a slender eelworm (possibly referable to *Plectus* sp.) and in intruding globose infective bodies from which assimilative hyphae have been extended through the animal. *E*, Portion of prostrate hypha with a two-celled adhesive outgrowth on whose distal cell a well developed eelworm is held affixed by means of a visible cushion of glutinous material; from the distal cell a globose infective body has been intruded that has given rise to growing assimilative hyphae. *F*, Portion of prostrate hypha with a two-celled adhesive outgrowth whose proximal segment is holding captive a slender eelworm; from a globose infective body intruded into the animal assimilative hyphae are being extended through its fleshy body. *G*, Portion of prostrate hypha with a two-celled adhesive outgrowth to whose basal segment a slender eelworm is held affixed; from the globose infective body intruded by the fungus a single assimilative hypha has been extended lengthwise through the animal's body. *H*, Portion of hypha bearing a three-celled adhesive outgrowth, whose proximal and distal segments have both been operative in capturing a slender eelworm; each of the two segments has

intruded a globose infective body from which one hypha is being extended forward and another backward through the animal's body. *I,* Prostrate hypha with a conidiophore from which a single conidium has been abjointed. *J,* Portion of prostrate hypha with an erect conidiophore, *a,* on which were produced the two conidia *b* and *c,* each of them shown with its basal end close to the position where it had been attached. *K,* Detached conidia showing usual variations in size and shape, together with variations in septation as follows: *a,* two septa present; *b–i,* three cross-walls present; *j–r,* four cross-walls present in symmetrical arrangement, two small cells being placed above and two small cells being placed below a large median cell; *s, t,* four cross-walls present, with the largest cell placed in penultimate position; *u–w,* five cross-walls present. *L,* Detached conidium that has put forth a unicellular adhesive outgrowth, *a,* from its basal cell. *M,* Detached conidium that has put forth one two-celled adhesive outgrowth, *a,* directly from its apical cell, and has extended from its tip a short stout hypha whereon is borne perpendicularly another two-celled adhesive outgrowth, *b. N,* Detached conidium that has put forth a two-celled adhesive outgrowth, *a,* from its tip, and from its basal cell has given off a unicellular adhesive outgrowth, *b,* as well as a four-celled adhesive outgrowth, *c.*

FIG. 8. *Dactylaria eudermata,* as found developing in nematode-infested agar plate cultures; drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A–D,* Portions of mycelial filaments, each bearing an adhesive hyphal network. *E,* Wide mycelial filament bearing a hyphal network, which, after being operative in capturing a sharp-tailed eelworm, *a,* intruded a globose infective body that has given rise to assimilative hyphae extending lengthwise through the fleshy interior; *b,* portion of the empty integument of a nematode that had been captured and depleted of its substance earlier; *c,* fusion of two assimilative hyphae. *F,* Portion of prostrate hypha with an erect conidiophore, *a,* from the tip of which has been abjointed a single conidium, *b;* the conidiophore being shown, from lack of space, in three parts whose proper connection is indicated by broken lines. *G,* Small portion of mycelial hypha with the proximal portion of a fallen conidiophore from which has been sent up a secondary conidiophore, *a,* that produced at its tip the conidium *b;* the conidiophore is shown, from lack of space, in three parts whose proper connection is indicated by broken lines. *H,* Detached mature conidia, *a–t,* showing usual variations in size and shape. *I,* Detached conidium that has sent up two slender aerial hyphae, *a* and *b,* from its parabasal cell, and one similarly slender aerial hypha, *c,* from its apical cell.

FIG. 9. *Dactylaria eudermata,* as found developing in nematode-infested agar plate cultures; drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* Portion of stout mycelial filament bearing a small adhesive network consisting of only two meshes. *B,* Portion of stout mycelial filament bearing a more extensive adhesive network. *C,* Successive stages in the closure of an adhesive hyphal bail: *a,* portion of mycelial filament bearing a completed hyphal bail to which a second hyphal bail is being added; as the elongating recurved uniseptate branch approaches distally the hyphal bail from which it originated the segment toward which its tip is directed extends a protuberance to meet it; *b,* same predacious apparatus 40 minutes later than in *a,* the protuberance and the tip of the recurving branch now being in contact; *c,* same predacious apparatus 20 minutes later than in *b,* showing fusion of the protuberance and the recurved branch; *d,* same predacious apparatus 2½ hours later than in *c,* showing one septum formed near place of union and another septum formed about 20 μ backward, so that the completed hyphal bail is divided into three segments of about equal length. *D,* Portion of stout mycelial filament bearing an adhesive hyphal network in which a sharp-tailed eelworm (possibly referable to *Rhabditis* sp.) has been captured; the network has intruded three globose infective bodies from which assimilative hyphae, numbering six in all, are growing lengthwise through the animal. *E,* Portion of prostrate hypha bearing an erect conidiophore, *a,* that bore three conidia, one having been abjointed from the axial tip, *b,* another from the tip of a primary branch, *c,* and a third from the tip of a secondary branch, *d;* the conidiophore being shown, from lack of space, in three parts whose proper connection is indicated by broken lines. *F,* Portion of prostrate hypha with an erect conidiophore, *a,* that bore four conidia, one having been abjointed from the axial tip, *b,* two from tips of primary branches, *c* and *d,* and a fourth from the tip of a secondary branch, *e;* the conidiophore being shown, from lack of space, in three parts whose proper connection is indicated by broken lines. *G,* Mature detached conidia, *a–y,* showing usual variations in size and shape. *H,* Empty envelope of conidium that has yielded its contents to the mycelial hypha with which it is fused.

FIG. 10. Drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A–F, Dactylaria eudermata* as found in nematode-infested agar plate cultures: *A,* Portion of mycelium bearing an adhesive network in which a sharp-tailed eelworm (possibly referable to *Rhabditis* sp.) has been captured; a globose infective body is being intruded into the living captive. *B,* Portion of hypha bearing an adhesive network in which a sharp-tailed eelworm has been captured; from the single large bulbous infective body intruded by the fungus, assimilative hyphae have been extended lengthwise through the animal. *C,* Portion of prostrate hypha whereon is borne a conidiophore, *a,* from the tip of which a single conidium, *b,* has been abjointed; the conidiophore being shown, from lack of space, in three parts whose proper connection is indicated by broken lines. *D,* Detached conidia, most of them (*a–e*) divided by three cross-walls, but one (*f*) containing only two cross-walls. *E,* Conidium germinating by production of two germ tubes, one being extended from the basal end, the other arising laterally from the parabasal segment. *F,* Conidium that after fusing with a mycelial hypha has been emptied through transfer of its contents to the mycelium. *G–M, Dactylaria eudermata* as found in pure culture on maizemeal agar: *G,* Detached conidia, showing variations in size and shape, together with variations in number and position of septa as follows: *a–c,* three septa present, with the largest cell placed in penultimate position as in most conidia produced in nematode-infested cultures; *d–m,* two septa present, with the largest cell being terminal in position; *n–q,* one septum present, with the larger cell being in distal position. *H,* Conidium that has put forth an aerial hypha from its small parabasal cell. *I,* Conidium that has germinated by extending a germ tube from its minute basal segment. *J,* Conidium germinating by

putting forth one germ tube from its small basal cell, another from its slightly larger parabasal cell, and a third from its small apical cell. *K*, Conidium germinating by putting forth one germ tube from its rather small parabasal cell, and another from its small apical cell. *L*, Conidium that after becoming fused with a mycelial hypha, was emptied by transfer of contents to the mycelium. *M*, Conidium that after becoming fused with a mycelial hypha, has been nearly emptied through transfer of contents to the mycelium. *N–P*, Conidial apparatus, presumably belonging to *Dactylaria eudermata*, that was found in a nematode-infested culture: *N*, Fallen conidium from which have arisen two conidiophores, *a* and *b*, each bearing three unicellular secondary conidia. *O*, Tip of a conidiophore sent up from a fallen conidium resembling that shown in *N*; from this tip and its lateral prolongation were abjointed seven unicellular secondary conidia. *P*, Detached unicellular secondary conidia, *a–c*.

FIG. 11. *Dactylaria haptotyla*, as found developing in nematode-infected cultures; drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, Portions of mycelial hyphae, each bearing a predacious organ consisting of a globose adhesive cell and a unicellular (*a*, *b*) or a bicellular (*c–o*) stalk. *B*, Portion of hypha with two predacious organs, *a* and *b*, both having two-celled stalks. *C*, Portion of mycelium with four predacious organs, *a–d*, each having a two-celled stalk. *D*, Portion of hypha with a predacious organ whose stalk, on repeated elongation, bore successively three adhesive cells, *a–c*, whereof the two formed earliest, *a* and *b*, have degenerated. *E*, Portion of hypha bearing besides the inactive predacious organ *a*, two other predacious organs that have operated jointly in capturing a nematode (*Panagrolaimus* sp.) and have each intruded a globose infective body from which assimilative hyphae are being extended; one of these active predacious organs is attached to the mycelium not only by its original stalk, *b*, but also by a supplementary connection, *c*, while the other organ seems attached only by a supplementary connection, *d*. *F*, Portion of mycelium with a predacious organ, *a*, that has been operative in capturing a small nematode (*Panagrolaimus* sp.) and has intruded a globose infective body from which assimilative hyphae are being extended. *G*, Portion of mycelium with a predacious organ that has been operative in capturing a nematode (*Panagrolaimus* sp.) and has intruded into the captive a globose infective body from which assimilative hyphae are being extended; the distal segment of the bicellular stalk, *a*, originally supporting the adhesive cell, having been damaged, a supplementary connection, *b*, has been formed uniting the proximal segment of the stalk with the adhesive cell. *H*, Portion of mycelium with a predacious organ that has been operative in capturing a nematode (*Panagrolaimus* sp.) and has intruded a globose infective body from which assimilative hyphae are being extended; the adhesive cell here is found attached not only by its original stalk, *a*, but also by a supplementary stalk, *b*. *I*, Portion of prostrate hypha with an erect conidiophore, *a*, whereon is borne a conidium, *b*. *J*, Portion of prostrate hypha with an erect conidiophore, *a*, bearing one conidium, *b*, on its axial tip, and another conidium, *c*, on the tip of a distal spur. *K*, Portion of prostrate hypha with an erect conidiophore from which a single conidium has been abjointed. *L*, Detached conidia, *a–z*, showing usual variations in size and shape; all containing four cross-walls,

with the middle cell, except in *z*, exceeding the other cells in size. *M,* Detached triseptate conidium that has put forth an aerial hypha. *N, O,* Detached quadriseptate conidia which have each put forth an aerial hypha. *P,* Detached quinqueseptate conidium that has put forth an aerial hypha. *Q,* Detached quadriseptate conidium that has put forth two aerial hyphae. *R,* Two conidia, *a* and *b,* that have become fused by means of an anastomosing connection.

FIG. 12. *Dactylaria haptotyla,* as found developing in nematode-infested cultures; drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* Portions of mycelial hyphae, each bearing a predacious organ, the adhesive cell of which in some instances, *a–d,* is supported on a unicellular stalk, and in other instances, *e–k,* is supported on a bicellular stalk. *B,* Portion of mycelial hypha with two predacious organs, *a* and *b.* *C,* Portion of hypha bearing a young predacious organ, *a,* and two fully formed predacious organs, *b* and *c;* the stalk in *b* is commonplace in being composed of two segments, whereas that of *c* is exceptional in consisting of three segments. *D,* Portion of hypha bearing besides an inactive predacious organ, *a,* another predacious organ that has operated in capturing a nematode (*Panagrolaimus* sp.) and has begun intruding a globose infective body into the animal; the adhesive cell of the active organ is supported by the original stalk, *b,* and also by the supplementary hyphal connection, *c.* *E,* Portion of hypha with an adhesive organ, *a,* that has operated in capture of a nematode (*Panagrolaimus* sp.) and has intruded into the animal a globose infective body from which assimilative hyphae are being extended; the adhesive cell here is connected with the parent hypha only by its original stalk. *F,* Portion of hypha with a predacious organ that has operated in capturing a nematode (*Panagrolaimus* sp.) and has intruded a small infective body from which assimilative hyphae are being extended; the adhesive cell here is attached to the parent hypha by its original two-celled stalk, *a,* and also by a supplementary connection, *b.* *G,* Portion of hypha with a predacious organ that has operated in capture of a nematode (*Panagrolaimus* sp.) and has intruded a globose infective body from which assimilative hyphae have been extended lengthwise through the animal; the adhesive cell having degenerated, its interior is in part occupied by a prolongation from the supplementary connection, *a,* which provides now the only communication between the assimilative hyphae and the external mycelium; at *b* is shown an anastomosis of an assimilative hypha with the tip of one of its branches. *H,* Portion of hypha with two predacious organs that have operated jointly in capturing a nematode (*Panagrolaimus* sp.), and have each intruded a globose infective body from which assimilative hyphae have been extended; the stalk *a* originally suporting one of the adhesive cells having degenerated distally, a supplementary connection, *b,* has been formed between the adhesive cell and the uninjured proximal segment of the stalk; *c,* supplementary hyphal connection that replaces the stalk originally supporting the other adhesive cell; *d, e,* anastomoses of assimilative hyphae. *I,* Portion of prostrate hypha with two conidiophores, *a* and *b,* which support aloft three (*c–e*) and four (*f–i*) conidia, respectively. *J,* Portion of mycelium with an erect conidiophore from which three conidia have been abjointed. *K,* Detached conidia, showing usual variations in size and shape, together with variation in septation as follows: *a, b,* three septa present; *c–x,* four septa present; *y, z,* five septa present. *L–N,* Quadriseptate conidia that have each put forth an aerial hypha, as usually, from the parabasal cell.

FIG. 13. *Dactylaria sclerohypha,* as found developing in nematode-infested agar plate cultures; drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* Portions of hyphae, *a–q,* each bearing a predacious organ with a two-celled stalk. *B,* Portion of hypha with two predacious organs, *a* and *b,* each having a two-celled stalk, though in *b* the proximal cell extends into the parent hypha. *C,* Portion of hypha with three predacious organs, two of them (*a, b*) having bicellular stalks, the other (*c*) having a unicellular stalk. *D,* Portion of mycelium bearing one predacious organ, *a,* with a two-celled stalk, and four other predacious organs, *b–e,* with unicellular stalks; *d* has been operative in capturing a nematode and has intruded a globose infective cell from which assimilative hyphae have been extended lengthwise through the animal; the predacious organ *e,* together with the two infective cells and the assimilative hyphae it extended into a captured nematode, is shown empty of protoplasmic contents. *E,* Portion of mycelium bearing two inactive predacious organs (*a* and *b*) with bicellular stalks, in addition to two predacious organs (*c* and *d*) that have each operated in capturing a separate eelworm and have each intruded into their captive a globose infective body from which assimilative hyphae have been extended; both *c* and *d* are attached mainly by supplementary connections; *e, f,* anastomoses of assimilative hyphae. *F,* Portion of hypha bearing a predacious organ with a unicellular stalk. *G,* Portion of hypha bearing one inactive predacious organ (*a*) with a unicellular stalk, and two predacious organs (*b* and *c*) that have each operated in capturing a separate nematode and have each intruded a globose infective body from which assimilative hyphae have been extended lengthwise within the animal; both active organs (*b* and *c*) show development of a supplementary hyphal connection; *d,* anastomosis of assimilative hyphae. *H,* Portion of hypha bearing an inactive predacious organ (*a*) with a unicellular stalk and also a predacious organ (*b*) that has operated in capturing a nematode and has intruded a globose infective body from which assimilative hyphae have invaded the animal lengthwise. *I, J,* Portions of mycelium each bearing a predacious organ with a two-celled stalk; the proximal cell in both instances extends into the parent hypha. *K,* Portion of mycelium bearing one predacious organ (*a*) with a unicellular stalk and another (*b*) with a long four-celled stalk. *M,* Portion of hypha bearing a predacious organ that has operated in capturing a nematode and has intruded a globose infective body from which assimilative hyphae have invaded the animal lengthwise; *a–c,* anastomoses of assimilative hyphae. *N,* Portion of mycelium bearing a predacious organ that has operated in capturing a nematode and has intruded a globose infective body from which assimilative hyphae have grown lengthwise in the animal; the empty distal portion of the two-celled stalk originally supporting the adhesive cell has been supplanted by a short supplementary hyphal connection. *O,* Portion of hypha with a predacious organ that after operating in the capture of a nematode has intruded a globose infective body from which assimilative hyphae have grown lengthwise through the animal; the

adhesive cell here is attached by its original two-celled stalk, *a,* and also by a supplementary connection, *b.*

FIG. 14. *Dactylaria sclerohypha,* as found developing in nematode-infested agar plate cultures; drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* Portion of prostrate mycelium from which has been sent up an erect conidiophore, *a,* bearing three conidia, *b–d.* *B,* Portion of mycelium with a prostrate hypha representing apparently a fallen conidiophore, *a,* which has sent up from a proximal segment an erect secondary conidiophore, *b,* bearing eight conidia, *c–j,* in loose capitate arrangement. *C,* Erect conidiophore bearing five sterigmata in rather close arrangement, from each of which a conidium has been abjointed; the branching hyphae at the base consist largely of proximal portions of older conidiophores that have fallen over on to the substratum. *D,* Prostrate mycelial hypha with proximal portions of both the fallen primary conidiophore *a,* and the fallen secondary conidiophore *b;* from the latter is shown arising a tertiary conidiophore, *c,* with five denuded sterigmata. *E, F,* Distal portions of denuded conidiophores provided with sterigmata in numbers of three and five, respectively. *G, H,* Random assortment of detached conidia, *a–z,* showing usual variations in size, shape, and septation. *I,* Detached conidium that has put forth a predacious organ from its distal end, and an erect aerial hypha from its parabasal cell. *J,* Detached conidium that has put forth a predacious organ from its median cell and an erect aerial hypha from its parabasal cell. *K,* Detached conidium that has put forth an erect aerial hypha from its parabasal cell. *L,* Two detached conidia, *a* and *b,* united through vegetative fusion, that have sent up the conidiophores *c* and *d,* respectively, which bear the conidia *e* and *f,* respectively. *M,* Cuticle of a captured nematode within which the assimilative hyphae and the globose infective body have become conspicuously indurated. *N,* Portion of an extensive branching hyphal system consisting mostly of somewhat thick-walled, enlarged, globose, indurated cells (or chlamydospores) with globuliferous contents.

FIG. 15. *Gonatobotrys simplex,* drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* Portion of prostrate hypha with an erect conidiophore bearing five conidial clusters *a–e;* drawn from material that developed on a tomato leaf in a moist chamber at 18° C. *B,* Denuded terminal portion of a conidiophore formed on a tomato leaf in a moist chamber at 18° C.; the conidiophore shows four noticeably swollen nodes, *a–d,* beset with truncate protuberances from which conidia have been abjointed. *C,* Denuded conidiophore with a swollen tip, *a,* bearing truncate protuberances from which conidia were abjointed; clusters of conidia likewise were abjointed from the swollen tips, *b* and *c,* of the two lateral branches; to the prostrate hypha from which the conidiophore arises is attached a globose unicellular branch, *d,* and a bicellular branch, *e;* drawn from a pure culture of the muskmelon strain on maizemeal agar; from lack of space the conidiophore is shown in three parts whose proper connection is indicated by broken lines. *D,* Portion of primary conidiophore, *a,* from which a secondary conidiophore, *b,* has been sent up; one denuded sporiferous node, *c,* is shown in the primary conidiophore, and another, *d,* in the secondary conidiophore; the swollen tip *e* of the secondary conidiophore is beset with conidia in a very early stage of development; drawn from a pure culture of the muskmelon

strain on maizemeal agar. *E,* Portion of primary conidiophore, *a,* from which a secondary conidiophore, *b,* has been sent up; a denuded sporiferous node, *c,* is shown in the primary conidiophore, and another, *d,* in the secondary conidiophore; the swollen tip, *e,* of the secondary conidiophore is beset with young growing conidia; produced on a tomato leaf in a moist chamber at 18° C. *F* (*a–z*), *G* (*a–o*), Detached conidia showing usual variations in size and shape; taken from a tomato leaf that had been kept in a moist chamber at 18° C. *H* (*a–z*), *I* (*a–h*), Detached conidia taken from a muskmelon leaf that had been kept in a moist chamber at 18° C. *J,* Detached conidia, *a–o,* as found in a pure maizemeal-agar plate culture of the muskmelon strain seven days after inoculation. *K,* Two uniseptate conidia, *a* and *b,* found among a multitude of non-septate conidia produced by the fungus on a tomato leaf in a moist chamber at 18° C. *L,* Portion of mycelium from a maizemeal-agar culture, showing three unicellular globose branches, *a–c,* and a bicellular branch, *d.* *M,* Portion of mycelium from a maizemeal-agar culture, showing ten unicellular globose or ellipsoidal branches, *a–j,* and two bicellular branches, *k* and *l.* *N,* Portion of mycelium from a maizemeal-agar culture, showing five unicellular globose or ellipsoidal branches, *a–e,* and a bicellular branch, *f.* *O,* Portion of mycelium from a maizemeal-agar culture, showing four unicellular globose branches, *a–d,* borne laterally on a longish hypha, and two others that are borne separately on a unicellular branch, *e,* and a bicellular branch, *f.* *P,* Portion of mycelium from a maizemeal-agar culture, showing eight short unicellular branches, *a–h,* and a somewhat longer unicellular branch, *i.*

NEW OR NOTEWORTHY FUNGI FROM MT. RAINIER NATIONAL PARK *

ALEXANDER H. SMITH AND DANIEL E. STUNTZ

(WITH 21 FIGURES)

As part of a general program involving a manual on the gill-fungi of our western United States, which has occupied the senior author for a number of years, the collecting season of 1948 was spent at Mt. Rainier National Park. The personnel of the expedition included Mr. Henry Imshaug, the senior author's assistant, and Mr. Emory Simmons, who was collecting Pyrenomycetes for Prof. L. E. Wehmeyer. The junior author joined the party early in July. Laboratory space and living quarters at Longmire were provided for us by the Park authorities. The entire personnel of the expedition is greatly indebted to Mr. John C. Preston, Superintendent, to Mr. Russell Grater, Naturalist, to Mr. Merlin K. Potts, Assistant Naturalist, as well as to Dr. E. T. Bodenberg, Ranger Naturalist, for the facilities received and also for many helpful suggestions and courtesies which added greatly to the efficiency with which we were able to carry out our studies.

We began work during the last week in June and continued until the onset of cold weather and snow at the end of September. The season was an unusual one in that, because of frequent rains, the woods remained moist all summer. Fleshy fungi were fairly abundant until near the middle of September, at which time, because of a short period without rain, the production of fruiting bodies slackened appreciably. Although ample rain came after that date, relatively cool temperatures, apparently, retarded any further development of fleshy fungi. Although the fall mushroom

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flora, characteristically the most prolific one of any season in this area, was exceptionally poor, the summer flora was exceptionally good. Since the summer agaric flora of the Cascades as a whole is the most difficult to obtain, we felt very fortunate indeed. Without a doubt these circumstances are largely responsible for the considerable number of new and interesting fungi found in the area. Both of us have collected extensively in the Pacific Northwest, but our activities have been limited mostly to spring and fall collecting.

The area which we covered within the Park was relatively limited since the richness of many spots required that they be visited repeatedly. The lower Nisqually River, below the junction of Kautz Creek, was one such area, but the lower Tahoma Creek Valley, from where the creek crosses the road to Longmire on up to the Tahoma Creek Forest Camp, was the most outstanding. A majority of our collections came from these two areas. A great deal of time was spent working the higher country, but the fungous flora which came out as the snow melted was limited in extent and persisted until snow came again in the fall. We believe that this represented an unusual fruiting pattern which was dictated by unusual weather conditions. Only one trip was made up the Carbon River Valley, and no collecting was done between the Carbon River and Round Pass. Mr. Imshaug spent the early part of the season collecting at and above Ohanapecosh Hot Springs. From this it can be readily seen that in spite of making over 3500 collections of fleshy fungi, an overall picture of the Park flora in this group was not obtained.

Because we were interested particularly in *Psathyrella* and *Inocybe* respectively a special study was made of each genus. Fortunately both genera were well represented in the flora. The following species represent a few of the more outstanding discoveries. Additional data on fungi in other genera will be published by us at a later date, or by those to whom material has been sent for critical study.

Color names cited from Ridgway (**1912**) are enclosed within quotation marks. Those cited from Maerz and Paul (**1930**) are indicated as follows: raw umber 15A12, etc.

Clitocybe subvelosa sp. nov. *Fig. 21c*

Pileus 3–9 (10) cm. latus, subplanus, margine involutus, demum subinfundibuliformis, viscidus, aurantio-cinnamomeus vel incarnato-cinnamomeus, odor farinaceus, sapor praerancido-farinaceus; lamellae avellaneae vel incarnato-avellaneae demum subfuligineae, confertae, late decurrentes; stipes 6–9 cm. longus, 8–15 mm. crassus, aequalis vel deorsum clavatus, solidus, intus avellaneus, externe "pale vinaceous fawn" vel "vinaceous buff" et fibrillosus, sursum fibrilloso-annulatus; sporae 8–10.5 × 5–5.5 μ, ovatae vel subellipticae.

Pileus 3–9 (10) cm. broad, disc flat and margin inrolled at first, expanding to shallowly depressed on disc and with spreading or arched margin, surface viscid, toward the margin with streaks of agglutinated fibrils, edge at first fringed with veil remnants, disc glabrous and in some watery-spotted, color "orange-cinnamon" or darker at first, margin "light pinkish cinnamon" colors duller in age; flesh "pale pinkish buff" except near gills where it is watery avellaneous, odor farinaceous, taste intensely bitter-farinaceous (very rancid); lamellae "avellaneous" to "vinaceous buff" young, becoming "wood brown" to subfuligineous in age, close to crowded, broad, decurrent, not separable, often forked, edges even; stipe 6–9 cm. long, 8–15 mm. in diam., equal to clavate, solid, more or less avellaneous in cortex, surface "pale vinaceous fawn" to "vinaceous buff" and appressed fibrillose, cottony at base, with a thin fibrillose annular zone of partial veil fibrils near apex. Spores 8–10 × 5–5.5 μ, white in deposit, ovate pointed at maturity, nonamyloid, smooth; basidia 36–40 × 5–7 μ, four-spored, hyaline in KOH, yellow in iodine; pleurocystidia and cheilocystidia none differentiated; gill trama (as seen in sections of fresh material) distinctly of interwoven hyaline filaments; pileus trama homogeneous beneath a distinct gelatinous pellicle composed of narrow (2.5–4 μ) orange-brown hyphae as seen in water mounts of fresh material, hyaline revived in KOH; clamp connections present.

Habit, habitat and distribution: gregarious under alder and conifers, lower Tahoma Creek, September 12. Coll. by D. E. Stuntz (Sm. *31182*—**type**).

Discussion: The intense taste, incarnate-orange color, viscid cap, fibrillose veil, and the change in the color of the gills from youth to age amply distinguish this fungus from other species in the genus. It appears to be most closely related to *C. gomphidioides* in all characters except the presence of a distinct fibrillose veil and in the shape and size of the spores. The differences in the spores are shown (FIG. 21*c*, *d*). Drawings for both species were made from the types. My first impression of this fungus was that it belonged in *C. gomphidioides* and that I had failed to note the veil in the Olympic specimens. However, a critical comparison of the dried specimens and the photographs of each made at the time of collection shows my first impression to have been false. When one considers other instances of parallel species, largely distinguished by the presence of a veil in one and its absence in the other, it is evident that the logical procedure is to regard *C. subvelosa* as distinct from *C. gomphidioides.* It should be kept in mind that for years it was questioned whether or not *Armillaria mellea* and *Clitocybe tabescens* were distinct. Rhoades (1945) has clearly established that they are. Another parallel situation is that of *Hygrophorus erubescens* (veil-less) and *Hygrophorus purpurascens* (with a fibrillose veil). Since both *C. subvelosa* and *C. gomphidioides* bear a striking resemblance to species of *Hygrophorus* possessing divergent gill trama, a careful study of the arrangement of the hyphae of the gill trama in *C. subvelosa* was made from both young and old caps in the fresh condition. In both stages it was found to be interwoven.

Cortinarius rainierensis sp. nov. *Fig.* 1

Pileus 3–8 cm. latus, obtusus vel conicus demum campanulatus vel subplanus, siccus, squamulosus, "russet" vel "tawny," saepe "Sanford's brown"; odor raphanaceus; lamellae fulvae demum subferrugineae, latae, subdistantes, venosae; stipes 5–8 (10) cm. longus, 10–12 (15) mm. crassus, farctus, deorsum demum obscure ferrugineus, fibrilloso-annulatus; sporae 9–11 × 6.5–8 µ, late ovatae.

Pileus 3–8 cm. broad, obtuse to convex, expanding to conic campanulate, conic umbonate, obtusely campanulate or broadly convex, sometimes nearly plane in age, surface dry, innately fibrillose-squamulose over disc or nearly to margin, "russet" on disc and "tawny" over marginal area, when fruiting luxuriantly evenly rich tawny to "Sanford's brown," unchanging or merely slightly darker in extreme age; flesh rather thick in disc, thin away from the stipe, pale buff, odor of radish, taste similar but very slight; lamellae "ochraceous tawny" young, soon "tawny" and remaining so, broad, subdistant, adnexed, thick and intervenose, edges even; stipe 5–8 (10) cm. long, 10–12 (15) mm. thick, stuffed with a pale buff pith, cortex pale to dark tawny, darkening in base to Sanford's brown or darker where handled, mycelium white.

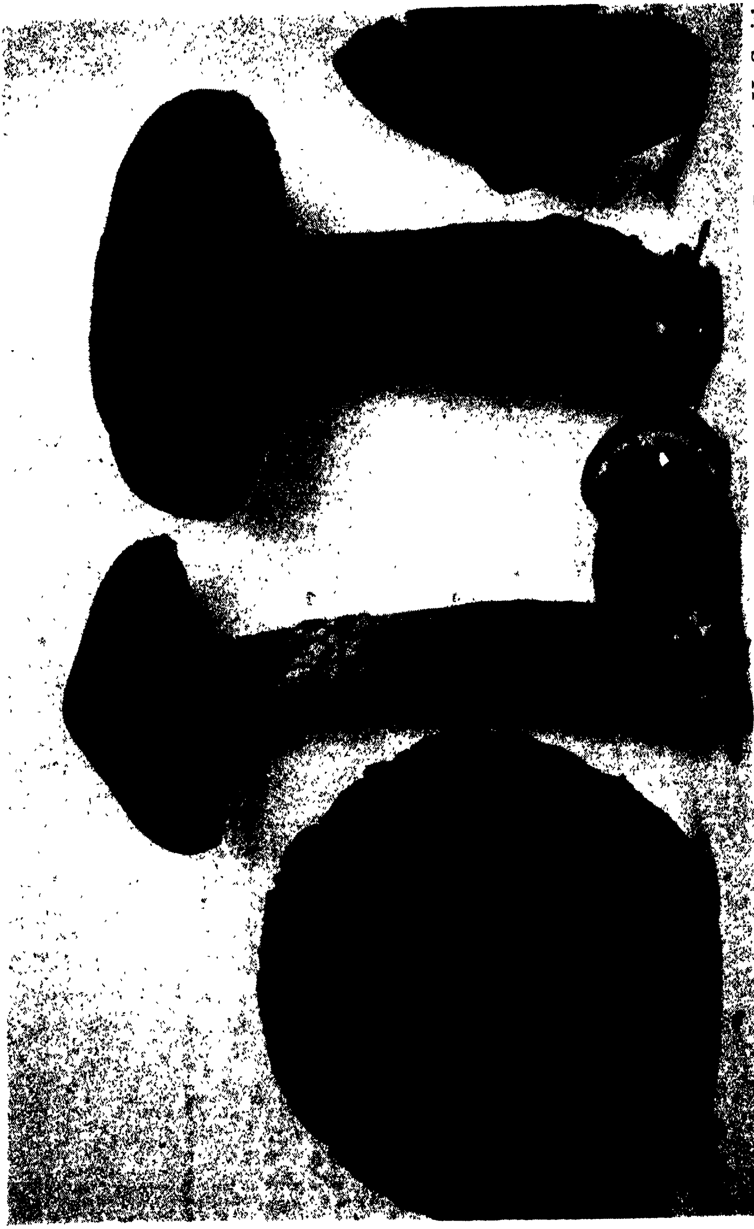

Photo A. H. Smith

FIG. 1. *Cortinarius rainierensis.* × 1.

Spores dark rusty brown in KOH, 9–11 × 6.5–8 μ, broadly ovate, surface roughened; basidia four-spored, 38–52 × 8–10 μ, hyaline in KOH (many brownish collapsed basidia also present); pleurocystidia none; cheilocystidia basidia-like but smaller, 30–40 × 5–8 μ, narrowly clavate to subcylindric and hyaline or nearly so; gill trama parallel or nearly so, yellowish (in thin sections) to rusty reddish (in thick sections) in KOH, pileus characterized by fascicles of rusty brown to yellow-brown (in KOH) hyphae projecting to form squamules, pigment incrusted on the wall but deposits not heavy, clamp connections present.

Habit, habitat and distribution: Scattered to gregarious on humus in virgin mossy Douglas fir-hemlock forests. August 18, lower Tahoma Creek (Sm. *30281*—**type**). The first collection to come to our attention was one by Stuntz (Sm. *29504*) at Barlow Pass in the Washington Cascades July 26, 1948. Numerous collections, however, were made in the Park as follows: Imshaug *2126*, Nisqually River below Longmire, August 30; Imshaug & Smith (Sm. *29806*), lower Kautz Creek, August 4; Sm. *30535*, lower Tahoma Creek, August 23; Sm. *31083*, September 9; Sm. *31632*, Longmire, September 22.

Discussion: This is a striking species and one which can be recognized at a glance. At maturity it is practically unicolorous. The color and dry scaly cap are sufficient for field identification. In the herbarium it is macroscopically indistinguishable from *C. distans* var. *olympianus* Smith. However, the latter has distinctly narrower spores, 5–6 μ as contrasted to 6.5–8 μ, which makes collections easily recognizable when dried. In the fresh condition *C. distans* var. *olympianus* is hygrophanous and when moist has a faintly striatulate margin. *C. rainierensis* appears to be related to *C. annulatus* by the odor of radish, scaly cap and fibrillose ring which is usually present on the stipe, but differs in the much larger spores. Among the European species it appears close to *C. tophaceus* but differs in the rusty brown rather than ocher-yellow color. *C. fulvaureus* Henry and *C. speciosus* Favre, two recently described species, appear to be somewhat similar in stature. However, the former differs in its spore characters and the latter in its much more brilliant colors as clearly indicated by Favre (**1948**) both in his description and illustration.

CORTINARIUS SUBTORTUS Fries *Figs.* 2; 3*a*

Pileus 3.5–5 cm. broad, buttons broadly convex to flattened and the margin inrolled, expanding to broadly convex or nearly plane, glabrous, glutinous to viscid but soon dry and shining, some with agglutinated fibrils along margin, variously colored "Isabella color" to "honey yellow" and finally near "Sayal brown" or mottled "dark olive-buff" on an "Isabella color" background, some "light brownish olive" over all, occasionally splashed with fulvous in age; flesh mottled "snuff brown" and "buffy brown," becoming honey-yellow or more fulvous in age, taste mild to slightly bitterish or with bitterish aftertaste, odor faintly pungent but hardly distinctive; lamellae "citrine drab" to "buffy brown," in some "brownish olive," "light brownish olive" or "Isabella color," slowly changing to cinnamon brown or more fulvous (near "raw sienna"), depressed-adnate to broadly adnate or subdecurrent, narrow and intervenose but finally moderately broad, edges even; stipe short, 3–5 cm. long, (4) 6–10 (15) mm. thick, clavate, becoming subequal, stuffed with a white pith, cortex avellaneous, surface olive-buff from a dense coating of fibrils, with a median to superior fibrillose zone and at times the sheath breaking up into obscure zones, silky and sordid olivaceous above at first, in age paler and more yellowish (near "honey color"), a few (in Sm. *27980*) with an obscure violaceous tinge in apex.

Spores 7–9 × 6–7 μ, broadly ovate-pointed to subglobose, rusty brown in KOH, slightly roughened; basidia 27–33 × 7–8 μ, four-spored, yellowish in KOH; pleurocystidia abundant, 50–80 (100) × 8–10 μ, in water mounts of fresh material often with an incrusting sheath, smooth as revived in KOH, hyaline and thin-walled, narrowly ventricose to subcylindric or neck tapered, wavy, and with acute apices; .cheilocystidia similar to pleurocystidia but shorter; gill trama parallel, pale yellow in KOH; cuticle of cap a gelatinous pellicle, the hyphae yellowish.

Habit, habitat and distribution: Gregarious to subcespitose under conifers, especially around very decayed stumps and logs, lower Nisqually above junction with Kautz Creek, and along highway near Tumtum Mt., Sept. 22 (Sm. *31608*), and Sept. 23 (Sm. *31634*). The senior author first collected this species on the east fork of the Salmon River, Mt. Hood, Oregon, at an elevation of about 4500 ft., Oct. 20, 1947 (Sm. *27980*).

Discussion: This species is very distinctive in the genus *Cortinarius* because of the presence of the greatly elongated pleuro-

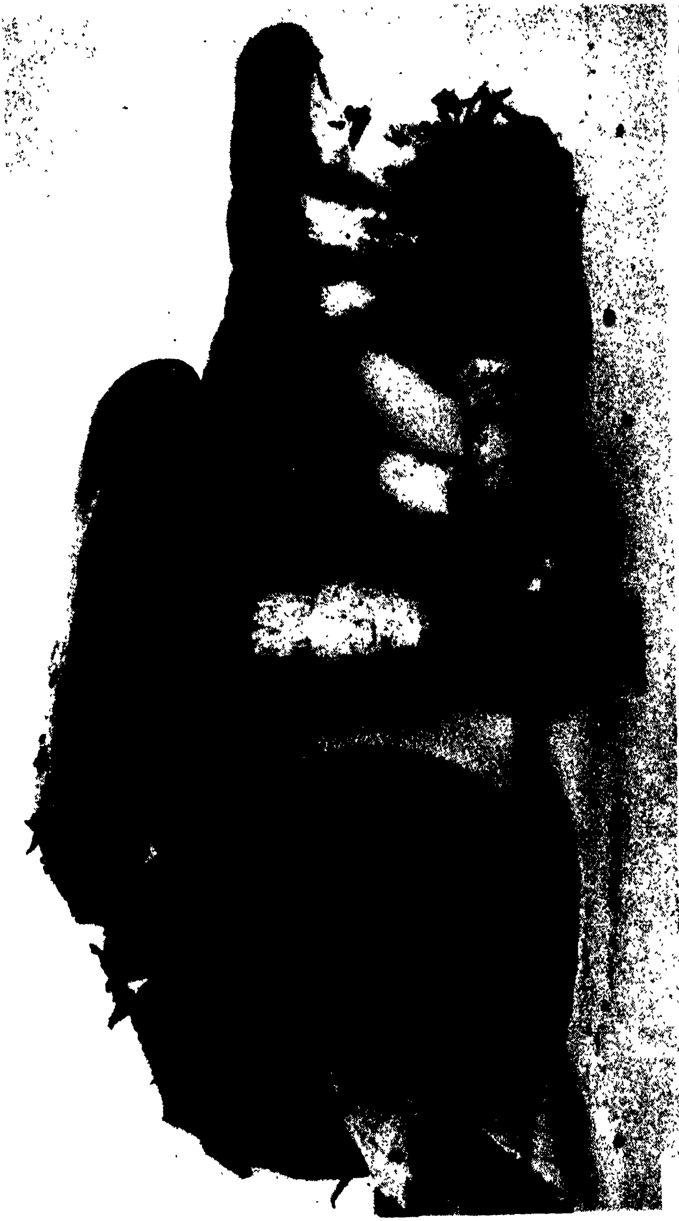

Photo A. H. Smith

FIG. 2. *Cortinarius subtortus.*

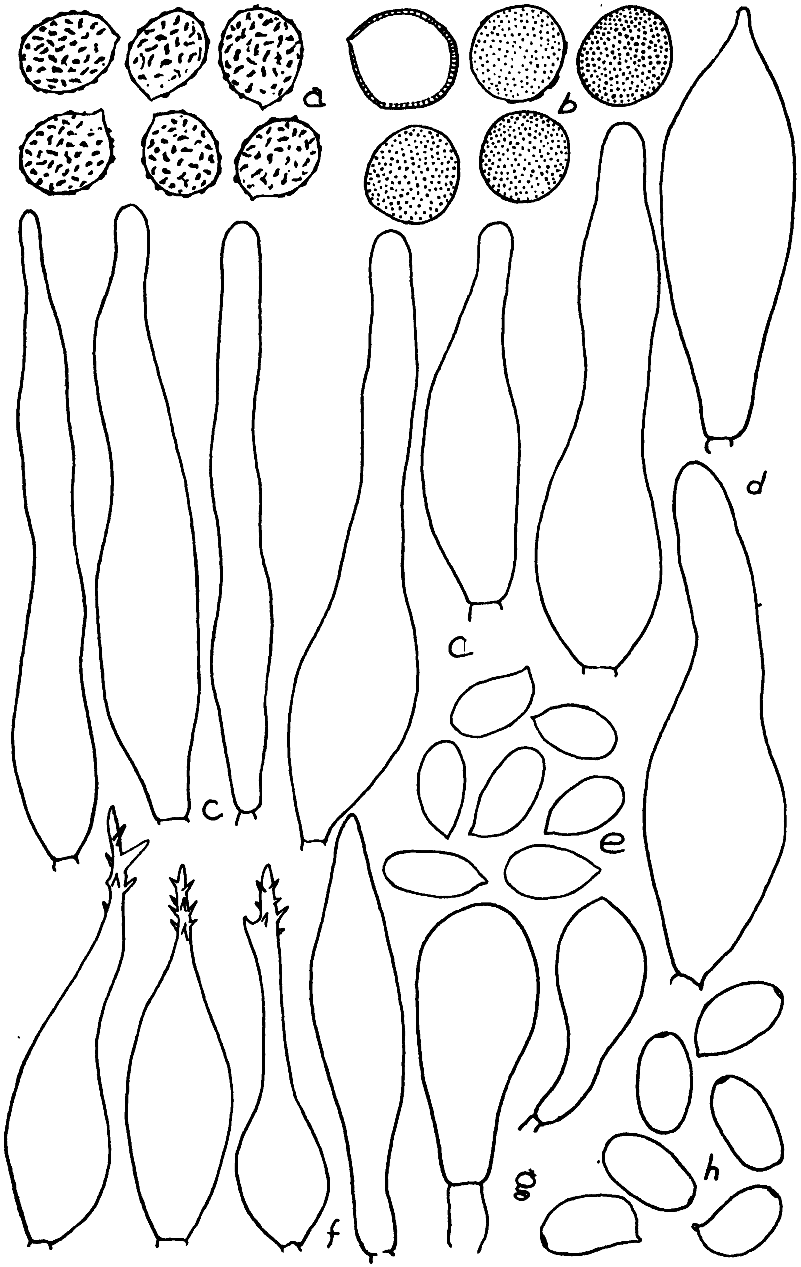

FIG. 3. Spores and cystidia of *Cortinarius, Mycena* and *Psathyrella.*

cystidia and a general resemblance to *C. infractus* in color. Maire et Kühner (1935) reported on this species as they found it in two different localities. Maire's collection was characterized by violaceous tints in stipe and gills. Coll. Sm. *27980* from Mt. Hood showed a slight tendency toward violaceous in the apex of the stipe. However, in the only extensive fruiting of this species we have observed here in North America, namely that made near Tumtum Mt. in the Park, the colors were more like those of Kühner's collection.

INOCYBE ARMORICANA Heim *Figs.* 4; 5*a*, *b*

Pileus 2.5–9 cm. broad, at first conic-campanulate, becoming expanded with a large prominent mammiform umbo, the margin soon plane and then finally a little uplifted; surface silky-smooth, lubricous when moist, drying silky-shining, often with a thin webby pallid coating of surface fibrils persisting as pallid patches at the center, toward the margin becoming more or less long-rimose, and at times regularly and conspicuously so, color at first "cadmium yellow" to "raw sienna," becoming "tawny" to chamois 11I5, 12E8, toast 13F8, or 13G9 at the margin; context 2–4 mm. thick off the disc, firm, white, unchanging on exposure, odor at first of green corn, then changing and becoming rather sweet and aromatic but still with an admixture of green corn; lamellae adnexed to almost free, broadly and shallowly rounded at the stipe, pointed at the margin, ventricose, widest toward the margin, 5–7 mm. broad, rather close, color white at first, becoming grayish olive (Arizona 13E6 to 14E6); stipe 4–12 cm. long, 5–13 mm. thick, terete or flattened, equal or in some a little incrassated downward, solid, the context firm and white to pallid yellowish, unchanging on exposure, usually becoming yellowish at the surface in age, base abruptly bulbous (submarginate) to clavate-bulbous and 13–20 mm. thick, surface satiny, glabrous to very slightly fibrillose-streaked, longitudinally striate, sometimes pruinate at the apex, color at first white, becoming pallid yellow all over, in age more or less flushed with darker shades of yellowish brown such as cinnamon 12E7 or 12F7, sometimes also with a subtle vinaceous cast. Spores 7–8.5 (10) × 5–6 μ, subreniform, smooth; pleurocystidia lacking; cheilocystidia 45–67 × 20–25 μ, broadly clavate.

Under conifers, Nisqually River at Southwest entrance to Park, A. H. Smith, July 28; lower Tahoma Creek, Smith and H. Imshaug, August 2 (St. *3813*); same locality, Smith, Imshaug

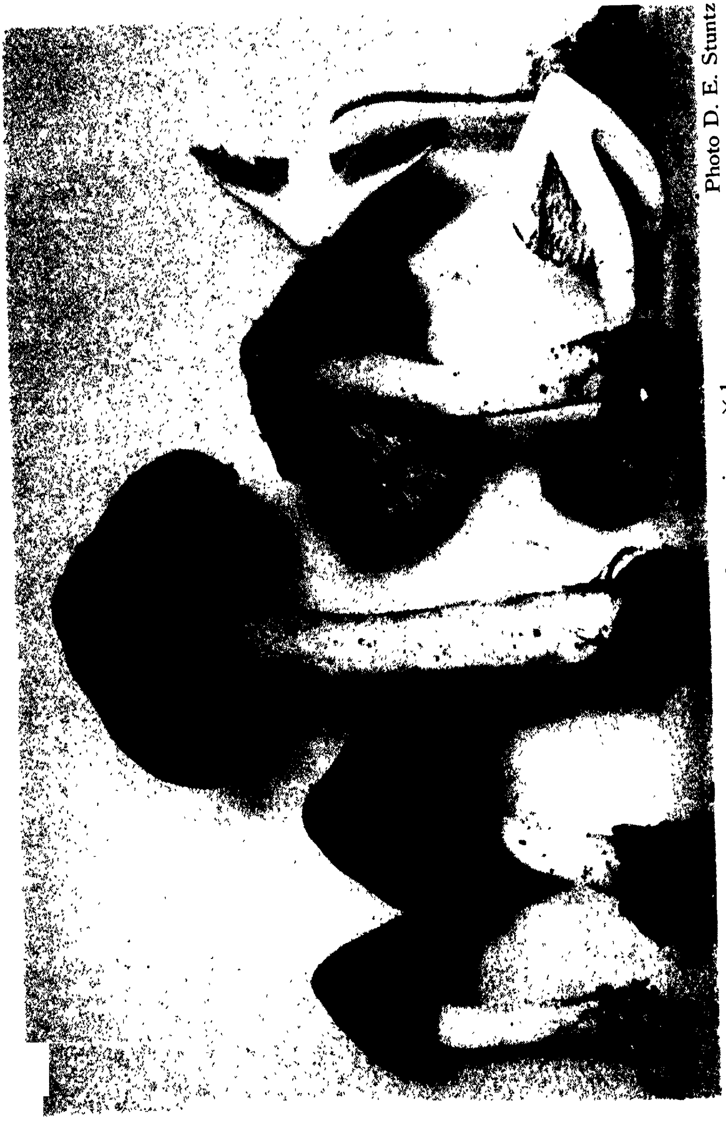

Photo D. E. Stuntz

FIG. 4. *Inocybe armoricana*, × 1.

and Grace Howard, August 3 (St. *3880*); same locality, August 9 (St. *3910*); lower Tahoma Creek, at the beaver dam, Elizabeth Knowles and D. E. Stuntz, August 15 (St. *3975, 3997*); same locality, August 21 (St. *4098*), September 12 (St. *4427*), Smith and Stuntz, September 14 (St. *4498*), Smith, Knowles, Margaret McKenny and Stuntz, September 19 (St. *4620*), September 21 (St. *4682*), September 22 (St. *4758*).

This is the first report of *Inocybe armoricana* Heim from North America. It was the dominant species of *Inocybe* in the lower Tahoma Creek area in late July and early August, and continued to appear sporadically in that area throughout the remainder of the collecting season. Its smooth yellow-orange to tawny pileus, rather massive white to pallid yellow stipe, and peculiar complex odor are quite constant characteristics by which it can be easily recognized. Some of the specimens are more than twice as large as the maximum dimension given by Heim (**1931**, p. 295), and in all the collections the strong green corn odor was much less modified by the subsequent development of a sweet or aromatic component than is indicated by Heim. Both these discrepancies seem to fall within the range of variability to be expected in the species, however, and scarcely could be considered sufficient grounds for excluding the collections from *I. armoricana*. Heim indicated (**1931**, p. *297*) a close relationship between this species and those related to *Inocybe jurana* Patouillard, especially *I. subrubescens* Atkinson. It might appear that an equally close or even closer relationship exists between *I. armoricana* and *I. sororia* Kauffman, since the principal differences between the two are those of pileus color and spore size, and the modification which the green corn odor of *I. armoricana* undergoes with the passage of time. Thus the *jurana* and the *fastigiata* species complexes may not be as unrelated as is commonly supposed.

INOCYBE FASTIGIATA (Fr.) Quél. f. ALPESTRIS Heim *Fig. 5c, d*

Pileus 4 cm. broad, 3.5 cm. high, conic-campanulate, sharply umbonate, with slightly incurved margin soon becoming long-rimose, color pale buff with an obscure tinge of tawny, near "cinnamon buff"; context about 2 mm. thick just off the disc, firm, white, unchanging on exposure, absolutely without odor; lamellae

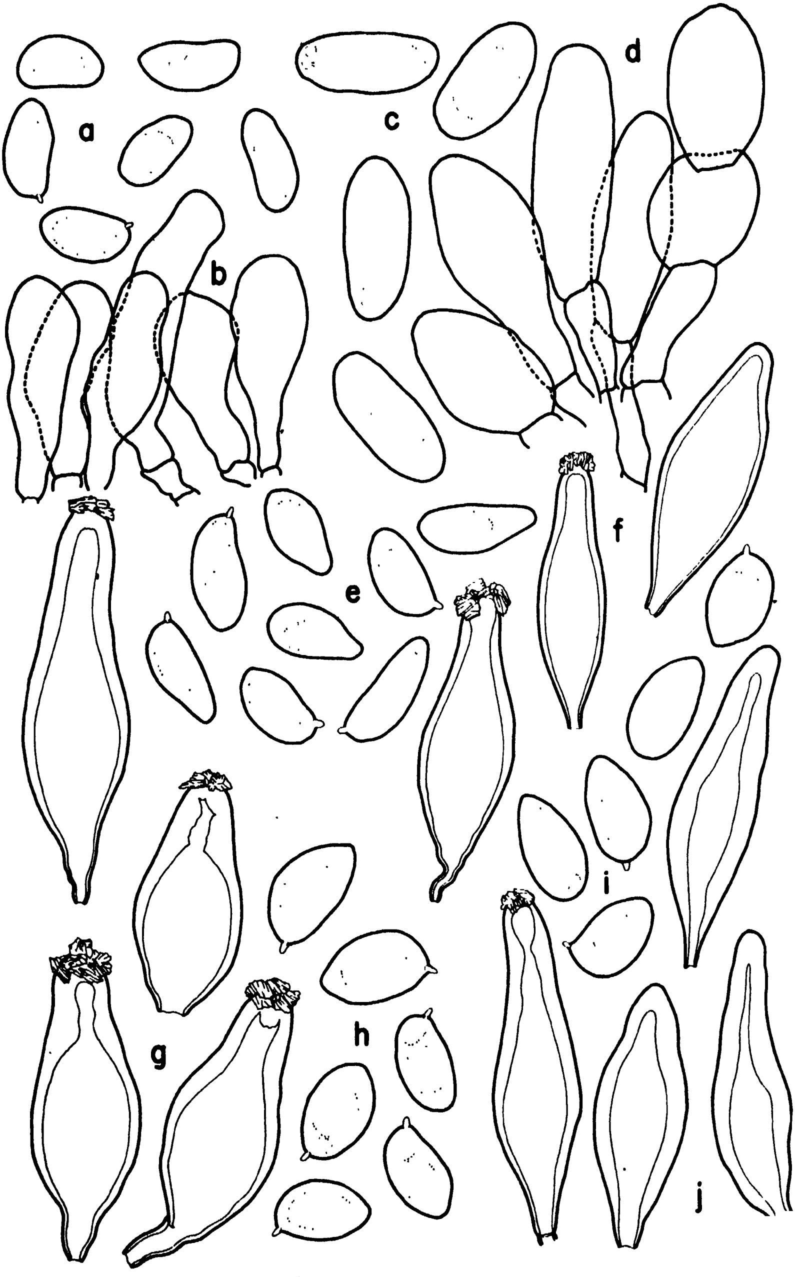

FIG. 5. Spores and cystidia of *Inocybe*.

adnate, plane both at stipe and margin, linear-oblong, narrow, 4 mm. broad, crowded, color pale grayish olive, with rather abundant brownish stains, the edges conspicuously white-fimbriate; stipe 9 cm. long, 6.5 mm. thick, terete, equal, flexuous, the base abruptly bulbous, 11 mm. in diameter, solid, context hard, white, unchanging on exposure, surface satiny, shining, sparsely white-fibrillose-streaked, color white, with a very faint tinge of yellow in some.

Spores 11.5–15 (16.5) × 6.5–7.5 μ, subcylindrical, occasionally subreniform; pleurocystidia lacking; cheilocystidia 36–37.5 × 16–26 μ, clavate, simple or often in short chains of two to three cells.

Under conifers, lower Tahoma Creek near the highway, A. H. Smith, July 29 (St. *3759*), and again in the same area, Smith and H. Imshaug, July 30 (St. *3796*).

Among the numerous varieties and forms of *Inocybe fastigiata* (Fr.) Quél. that occur in Washington, this one is easily recognized by its pallid yellowish buff pileus, long slender white stipe, and complete lack of any kind of an odor. It might be taken for a slender specimen of *I. sororia* Kauffm., but the lack of that species' characteristic strong green corn odor soon corrects that impression.

The use of the form name *alpestris* may require some explanation. It is fairly evident (see **1931**, p. 176, and the footnote on p. 392) that Heim did not really intend what he called "forme alpestris" to be given actual taxonomic status; yet he assigns varietal names to other collections (var. *arenicola*) some of which so closely resemble "forme alpestris" that one wonders why they were not included in it. The more luxuriant specimens, at least, of var. *arenicola*, are evidently more robust plants than "forme alpestris," are white rather than yellowish, and are said to have a detectable spermatic odor. Our collections are closer in these particulars to "forme alpestris" than to var. *arenicola*, and since they are readily distinguishable from typical *I. fastigiata*, the designation we have used for them seems justifiable.

INOCYBE GRISEO-LILACINA Lange *Figs. 5e, f; 6*

Pileus 1.5–3 cm. broad, obtusely campanulate, becoming expanded and plane or nearly so, with a rounded umbo, the margin persistently rounded, surface densely appressed-fibrillose, the cuticle becoming diffracted-scaly all over with furfuraceous squamules which are appressed to slightly recurved at the tip, color yellowish buff on the umbo (walnut 12E6 to bamboo 13I7), elsewhere dingy yellowish gray (Long Beach 12B4 or polo tan 13C6), context about 2 mm. thick off the disc, pallid, unchanging on exposure, odor spermatic but not strong; lamellae adnexed, somewhat angularly sinuate, bluntly pointed at the margin, subventricose, 2–3 mm. broad, moderately close, color at first pallid with a flush of violaceous, becoming dingy grayish (Aloma 13C7), finally quite dark olivaceous umber (15E12); stipe 2.5–4 cm. long, 3–5.5 mm. thick, terete, equal, usually flexuous, the base rounded-truncate, not at all bulbous; solid, the context pallid within, dull lilac at the surface, unchanging on exposure, surface densely appressed-fibrillose, the fibrils in tiny pointed longitudinally oriented streaks especially below, the apex pruinate and almost glabrous, color pallid grayish buff (near beige soiree 11B4) at the base, lilac above (bisque 11A3 to tawny birch 13A6), but made to appear considerably duller because of the dense coating of surface fibrils.

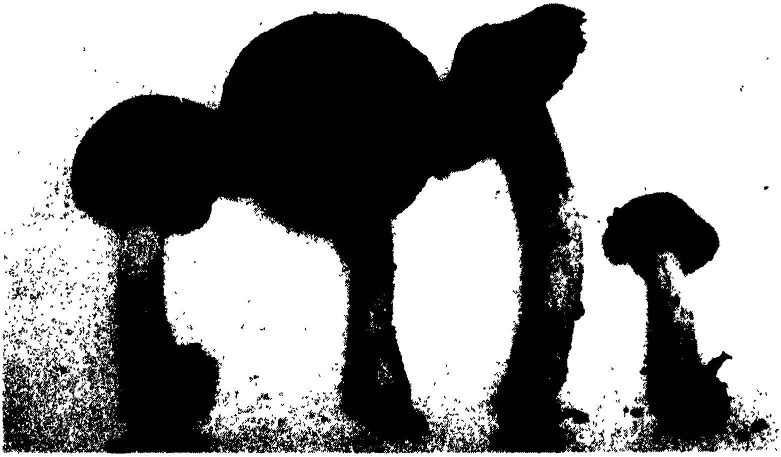

Photo D. E. Stuntz

FIG. 6. *Inocybe griseolilacina*, × 1.

Spores 8–9 (11) × 4.5–5.5 μ, inequilaterally amygdaliform; pleurocystidia 45–67 (80) × 14.5–18 μ, ventricose, with pedicel and slender neck, usually thick-walled; cheilocystidia of two kinds, some like the pleurocystidia in size and shape, the majority clavate to ovoid, thin-walled, 20–30 × 11–13.5 μ, densely clustered along the gill edge.

Under conifers, lower Tahoma Creek, A. H. Smith and H. Imshaug, July 30 (St. *3779*) ; Kautz Creek near the Nisqually River, Smith and Imshaug, August 4 (St. *3820, 3829*) ; lower Tahoma Creek, Smith and Imshaug, August 19 (St. *3925*) ; lower Nisqually River near Kautz Creek, Smith and Stuntz, August 14 (St. *3958*) ; Longmire, along the Trail of the Shadows, A. H. Smith, August 21 (St. *4051*) ; lower Tahoma Creek, Smith and Stuntz, September 8 (St. *4395*), September 12 (St. *4430*), and again, Smith, M. McKenny and Stuntz, September 19 (St. *4619*).

This *Inocybe* was found throughout the collecting season, but never in large numbers: all the collections cited except the one shown in figure 6 consist of but one or two carpophores.

Its rather pale yellowish gray squamulose pileus and fibrillose lilac stipe make it one of the more readily recognizable species of the *Inocybe obscura* group. Our specimens agree well with Lange's description and illustration (**1938**, p. 73, pl. 111–F), except for having a somewhat more robust stature. Heim considers Lange's species to be synonymous with *I. obscura* var. *violascens* (Quél.) Heim, but the spores he illustrates (**1931**, p. 256) for the latter differ enough in size and shape from those given by Lange (**1938**, p. 73, pl. 11–F) for *I. griseo-lilacina*, as well as from those of our own collections, to raise a reasonable doubt as to the advisability of merging the two.

Inocybe laetior Stuntz, sp. nov. *Figs.* 5*g*, *h*; 7

Pileus 2–3.5 cm. latus, e campanulatus expansus, umbonatus, sericeus, siccus, glaber, margine interdum subrimosus, centro fulvus vel badius, margine melleus vel olivaceo-brunneus; caro pallida, immutabilis, odor vix notabilis; lamellae adnexae, ventricosae, 6–7 mm. latae, ex pallidis olivaceo-brunneae; stipes 2.5–5 cm. longus, 2.5–5 mm. crassus, aequalis, basis leviter bulbosus vel non, omnino sericeus, subnitens, longitudinaliter striatus, laete salmoneus vel incarnatus, basis albus; sporae 9–11 (13) × 5.5–6.5 μ, leves, amygdaliformes; pleurocystidia (53) 60–80 × 20–30 μ, ventricosa.

Pileus 2–3.5 cm. broad, campanulate and umbonate, becoming expanded and finally plane or nearly so, with a low rounded umbo, the margin incurved at first, then slightly rounded, finally plane and more or less radially split, surface smooth, appressed silky, the cuticle dense, compact, remaining unbroken at the center, becoming more or less rimose toward the margin, usually not at all scaly but sometimes becoming so in dry weather, color at first uniformly

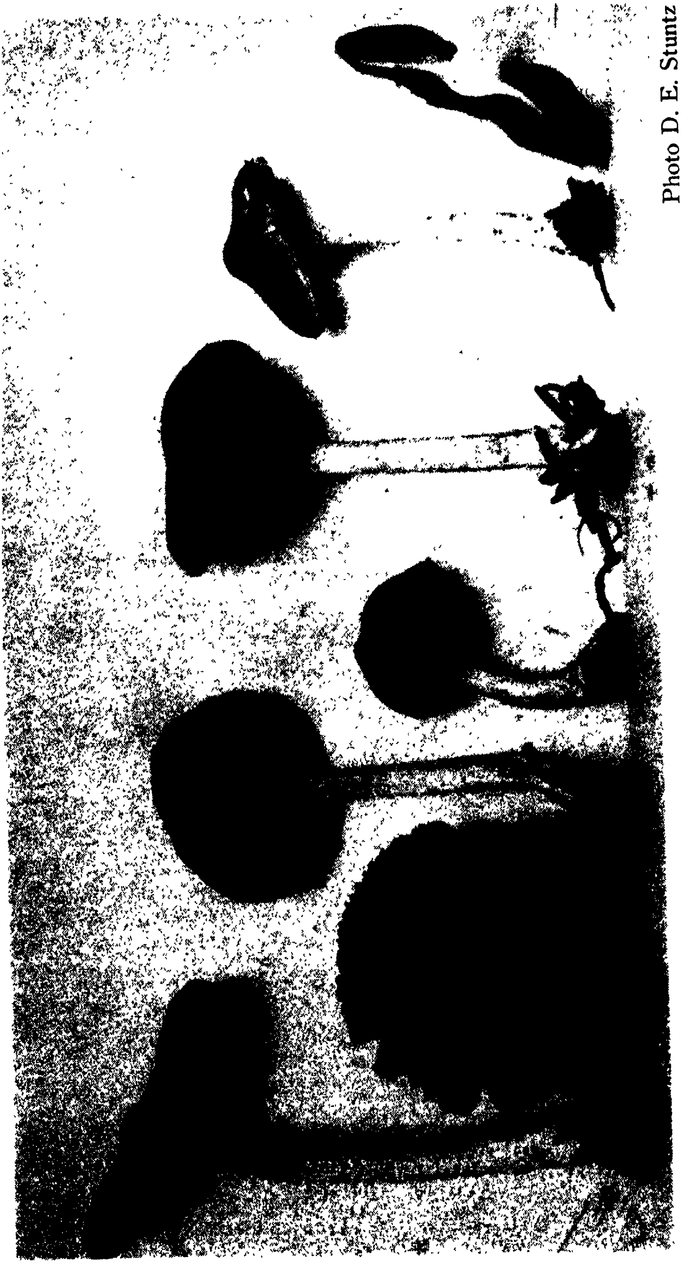

Photo D. E. Stuntz

FIG. 7. *Inocybe laetior*, ×1.

brown, then becoming brassy yellowish at the margin and the center remaining tawny brown, center gold brown 14G12, "auburn" or "Sanford's brown," margin at first 14H10 becoming centennial brown 13K8 to Isabella 13K7 or honey 12J6, rarely as dark as "Mars brown" in the center, and shading out through "Verona brown" to "Sayal brown" at the margin, marginal area often more or less virgate with the tawny cuticular fibrils of the center; context 2–3 mm. off the disc, white or pallid, unchanging on exposure, odor slight, spermatic-subraphanoid; lamellae adnexed or sometimes narrowly adnate, often minutely uncinate, broadly rounded at the stipe, bluntly rounded at the margin, asymmetrically ventricose, widest toward the margin, rather broad, 6–7 mm., not close, 30–35 reach the stipe, with about as many inserted, edges slightly serrulate in most specimens, color at first pallid, about sheepskin 11C3 or ecru 11C2, becoming 13I6 to 13J6 (pallid olivaceous), finally khaki 13J7 (dull olivaceous brown); stipe 2.5–7 cm. long, 2.5–5.5 mm. thick, terete or sometimes a little compressed, equal, the base usually slightly bulbous, solid, the context pale salmon-incarnate to yellowish incarnate, unchanging on exposure, surface satiny, somewhat shining, everywhere white-pruinate, finely but notably longitudinally grammate, the base densely white-myceloid, color bright salmon incarnate, often becoming paler with age (auteuil 11C7, 11B8, formosa 12A8, "light pinkish cinnamon," "pinkish cinnamon," or "vinaceous cinnamon," rarely as dark as "cacao brown," Mindoro 13A8 or 11D8), base persistently white.

Spores 9–11 (13.5) × 5.5–6 (6.5) μ, smooth, inequilaterally amygdaliform; pleurocystidia (53) 60–80 × 20–30 μ, ventricose, with very short pedicel and obtuse apex, thick-walled; cheilocystidia mostly like the pleurocystidia in size and shape but a few small clavate thin-walled cells also present.

Under conifers, lower Tahoma Creek, Smith and H. Imshaug (St. *3914*), August 18 (St. *4019*), September 12 (St. *4424*); A. H. Smith, September 12 (St. *4432*), September 12 (St. *4473*), Smith, September 14 (St. *4506*), September 18 (St. *4616*); Smith and Margaret McKenney, Tahoma Camp Grounds, September 20 (St. *4655*—**type**); Smith, lower Tahoma Creek, September 21 (St. *4668*); September 21 (St. *4675*); September 23 (*4710, 4712*).

This species can be recognized quite easily by its strikingly colored salmon-incarnate stipe and its pileus with red-brown center and brassy yellowish margin. These colors appear to be quite constant; of the twelve collections made, only one (*4712*) had both pileus and stipe somewhat darker than the others. The two species most closely related to *Inocybe laetior* are *I. obscura* var. *rubens* Heim and *I. subdestricta* Kauffman. From both of these *I. laetior* differs in its non-uniformly colored pileus, and especially in its much more brightly colored salmon-incarnate stipe. Its spores are about intermediate in size between those of the other two species, and its pleurocystidia resemble those of *I. obscura* var. *rubens* rather closely.

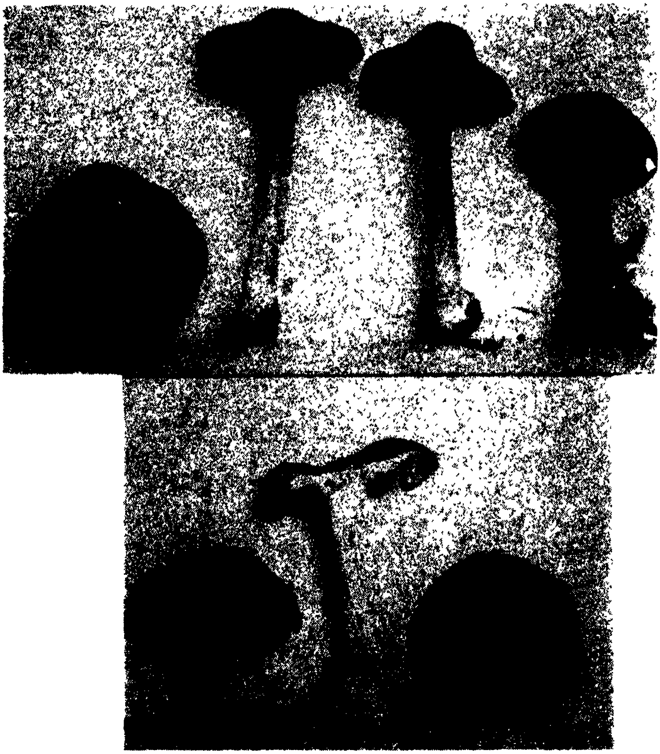

Photo D. E. Stuntz

FIG. 8. *Inocybe leiocephala*, × 1.

Inocybe leiocephala Stuntz, sp. nov. *Figs.* 5*i, j;* 8

Pileus 1.2–3.5 cm. latus, ex obtuse campanulatus expansus, umbonatus, glaber, centro obscure brunneus, margine castaneus vel rubrobrunneus; cutis compacta, hygrophana, nitens; caro firma, alba, immutabilis; odor nullus; lamellae adnatae, postice sinuatae, ventricosae, 4.5–5 mm. latae, ex pallidis olivaceo-brunneae; stipes 1.5–5 cm. longus, 2.5–5.5 mm. crassus, aequalis,

basis rare leviter bulbosus; omnino leviter pruinatus, subnitens, manifeste longitudinaliter striatus, incarnatus vel incarnato-cinnamomeus; sporae 8.5–12 × 5.5–6.5 μ, leves, inaequales; pleurocystidia 56–73 × 15–25 μ, ventricosa, muro valde crasso praedita.

Pileus 1.2–3.5 cm. broad, obtusely campanulate becoming expanded, with a low broad umbo and persistently rounded margin, surface smooth, glabrous, innately silky and shining, the cuticular layer dense, compact, rather hygrophanous, remaining unbroken or occasionally a little diffracted in places when dry, color "warm sepia" to "mummy brown" on the umbo, elsewhere varying from red-brown to dingy brassy yellow-brown, usually "russet," "auburn," "Sanford's brown," margin sometimes nearer "buckthorn brown," or sometimes the whole pileus uniformly "chestnut brown"; context 1–2 mm. off the disc, hard, white, unchanging on exposure, odor none, or very faintly spermatic; lamellae narrowly to broadly adnate, rounded-sinuate to emarginate, bluntly pointed at the margin, subventricose, 4.5–5 mm. broad, moderately close, color at first pallid, becoming olivaceous brown (Isabella 13K7, 14F7, syrup 14L8, finally darker, 15J10); stipe 1.5–5 cm. long, 2.5–5.5 mm. thick, terete or occasionally somewhat compressed, equal, base not bulbous, or rarely very obscurely so, solid, context pallid with a flush of incarnate, unchanging on exposure, surface satiny, shining, conspicuously longitudinally hygrophanous-grammate, pruinate all over, color incarnate, "pinkish buff" to "light pinkish cinnamon," becoming "pinkish cinnamon" to "cinnamon" (papyrus 12C7 or 13E7), finally "Sayal brown" in age.

Spores 8.5–10 (12) × 5.5–6.5 μ, somewhat variable in size, inequilateral; pleurocystidia 56–73 × 15–25 μ, ventricose with long neck and short pedicel, very thick-walled; cheilocystidia of two kinds, one the same size and shape as the pleurocystidia, the other thin-walled, clavate, 22–34 × 8–13.5 μ.

Habit, habitat and distribution: Under conifers, junction of Fish Creek with Tahoma Creek, Smith, Knowles and Stuntz, August 9 (St. *3929*); camp grounds, Longmire, Smith, August 31 (St. *4249*); in moss under Douglas fir near the inn, Longmire, September 1 (St. *4259*); same locality, September 24 (St. *4739*—**type**), and September 27 (St. *4816*).

Discussion: The very compact layer of cuticular hyphae gives the pileus surface of this species a glabrous, hygrophanous appearance unusual in *Inocybe,* and very similar to that of many of the small brown Cortinarii of the subgenus Hydrocybe. This characteristic, the rather dark red-brown pileus, small compact stature, incarnate stipe, and thick-walled pleurocystidia, are the principal distinguishing features. While the pileus usually has a very dark brown umbo and red-brown margin, it may be uniformly bay in color, or even almost a mahogany red, and is apt to become somewhat paler and more yellowish brown on drying. The most closely related species appear to be *Inocybe subdestricta* Kauffman, *I. obscura* var. *rubens* Heim, and *I. laetior* Stuntz, in diminishing order of resemblance. From all three of these *I. leiocephala* differs markedly in the nature of its pileus surface: it has smaller spores than *I. obscura* var. *rubens,* and differs further from *I. laetior* in the color of both pileus and stipe.

INOCYBE OBLECTABILIS Britzlmayr, fma. DECEMGIBBOSA Kühner
Fig. 9; 10*a, b*

Pileus 2–4 cm. broad, campanulate or conic-campanulate, expanding and becoming shallowly campanulate and finally almost plane, with a broad rounded umbo, margin incurved, usually becoming almost plane, often undulating and more or less broadly lobed, surface at first with a thin white webby coating of surface fibrils which persist for a time at the center as pale webby patches, finally vanishing entirely, cuticle silky smooth, lubricous when moist, shining when dry, remaining unbroken at the center, becoming conspicuously long-rimose toward the margin, color "Sayal brown" to "cinnamon brown," "Verona brown," 14G9, or burnt umber 15A12, the umbo often considerably paler (raffia 11E5 or pallid ivory); context 2–2.5 mm. thick off the disc, firm, white, unchanging on exposure, odorless; lamellae adnexed and uncinate, shallowly to deeply rounded-emarginate, pointed at the margin, oblong-subventricose, 4–6.5 mm. broad, moderately close, color at first pallid gray (putty 11B2), becoming darker and more olivaceous (grain 13B5 to cracker 13D6, finally 14I7 to maple sugar 14J8); stipe 2.5–7 cm. long, 3–7 mm. thick, terete or compressed, equal, the base with a conspicuous flat marginate bulb 7–13 mm. in diameter, solid, the context pallid or yellowish, usually with a flush of incarnate at the surface, unchanging on exposure, surface satiny, more or less shining, everywhere finely white-pruinate and conspicuously longitudinally hygrophanous-grammate, color pale yellow, usually with more or less of a flush of incarnate (10C5, or "light ochraceous buff," "pinkish buff," "light pinkish cinnamon," or "pinkish cinnamon"), the base persistently white.

Spores 9–11 × 6.5–8 μ, oblong, with an average of eight rather coarse nodules; pleurocystidia 45–67.5 × 13.5–20 μ, fusiform-

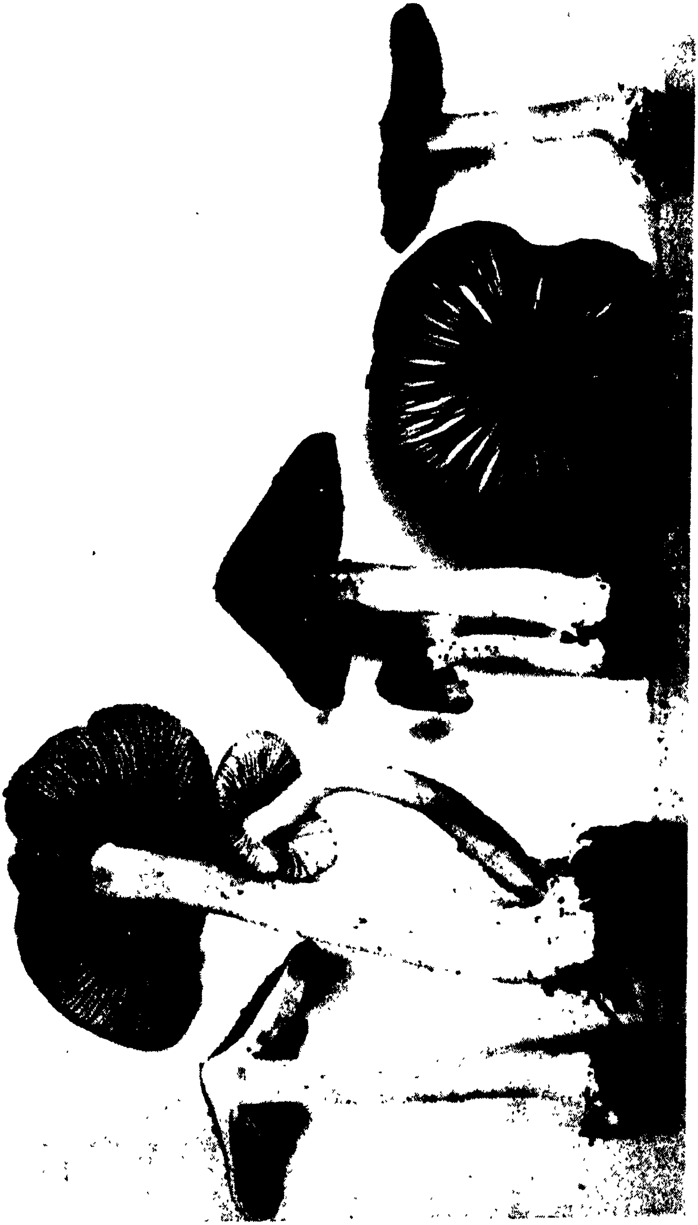

FIG. 9. *Inocybe oblectabilis* f. *decemgibbosa*, × 1. Photo D. E. Stu

ventricose with short neck and pedicel, moderately thick-walled; cheilocystidia like the pleurocystidia in size and shape, also thin-walled and clavate, 22.5–30 × 9–11.5 μ.

Habit, habitat and distribution: In a lawn under conifers, Longmire, August 4 (St. *3811*); under conifers, lower Tahoma Creek near the highway, Knowles and Stuntz, August 16 (St. *3980, 3981*); lower Tahoma Creek at the old campgrounds, Smith & Stuntz, August 20 (St. *4043*), same locality, August 22 (St. *4097*), September 8 (St. *4388*), September 12 (St. *4459*), and September 18 (St. *4612*); lower Tahoma Creek, conifer woods one half mile from the highway, September 21 (St. *4674*), same locality, Smith, September 21 (St. *4680*) and again September 23 (St. *4706*).

Discussion: Neither *Inocybe oblectabilis* Britz. nor its two forms have been reported previously from North America. The form *decemgibbosa,* however, is apparently not uncommon in Washington, having been collected by the junior author in several localities outside the Park. The incarnate color of its stipe is apt to vary from a slight flush to a decidedly salmon pink cast with different collections, and may even be lacking at the time the specimens are gathered, only to develop later after they have stood for a while. The other distinctive features (smooth, brown, rimose pileus and pallid grayish lamellae) are quite constant, however, and the ensemble of characteristics makes this an easily recognized *Inocybe*. The brown pileus and incarnate stipe readily separate it from *Inocybe praetervisa* Quél. and related species.

Inocybe picrosma Stuntz, sp. nov. *Figs.* 10*c, d;* 11

Pileus 1.5–4 cm. latus, e campanulato expansus et late umbonatus, siccus, primo sericeo-fibrillosus, postremo plus minusve squamuloso-diffractus, cremeus vel pallide luteus; caro pallida, immutabilis, odor valde specialis, aliquatenus raphaneus et resinosus, etiam acer; lamellae rotundato-adnexae vel anguste adnatae, ventricosae, 3.5–5 mm. latae, ex pallidis griseo-olivaceae; stipes 2.5–8.5 cm. longus, 3–7 mm. crassus, aequalis, basis plerumque abrupte bulbosus sed non marginatus, primo omnino albo-pruinatus, deorsum glabrescens, longitudinaliter striatus, pallide luteolus, aetate sordide brunneus vel avellaneus; sporae 8.5–10 × 5 μ, leves, amygdaliformes; pleurocystidia 40–60 × 16.5 μ, subclavata.

Pileus 1.5–4 cm. broad, campanulate and umbonate, becoming campanulate-expanded, the margin at first narrowly involute, long remaining broadly rounded, surface appressedly silky-fibril-

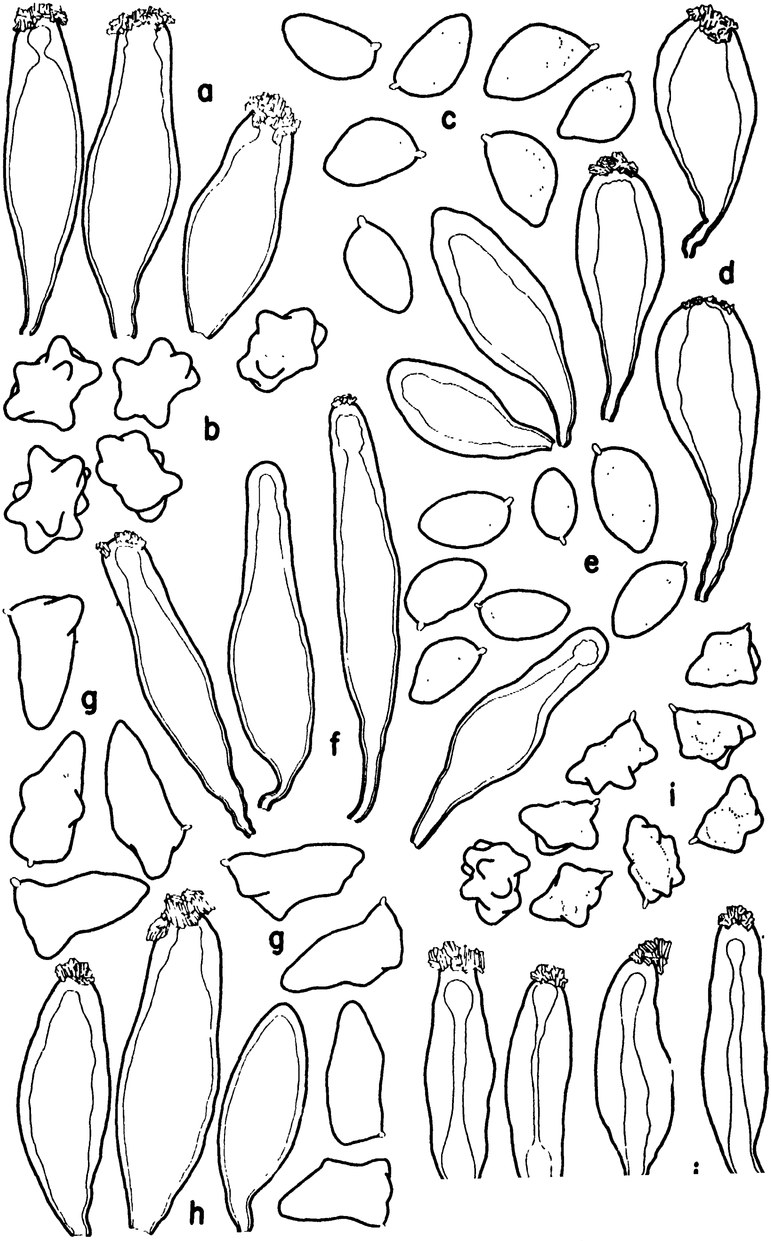

FIG. 10. Spores and cystidia of *Inocybe*.

lose, smooth, shining, some remaining so at all times, but the majority becoming more or less diffracted-scaly at least at the center and usually clear to the margin, color uniformly pale creamy yellow to pale buff, "cream buff," to "Naples yellow," 10F4, 11F5, or chamois 11I5, sometimes more or less flushed with "cinnamon buff" to "clay color" or even darker (macaroon 12H7) especially on the disc; context 1.5–3 mm. thick off the disc, firm but quite brittle, pallid to pallid yellowish, unchanging on exposure, odor very characteristic, not strong, but very penetrating, spermatic for an instant when the context is first exposed, but immediately becoming quite complex, predominantly a mixture of raphanoid and resinous with a trace of acetic acid, having a very decided pungency which quickly produces a tingling sensation in the back of the throat; taste a little astringent and acrid at first, soon becoming mild and almost sweet, but leaving an unpleasant aftertaste; lamellae adnexed to narrowly adnate, abruptly and deeply rounded at the stipe, broadly rounded at the margin, ventricose, moderately broad,

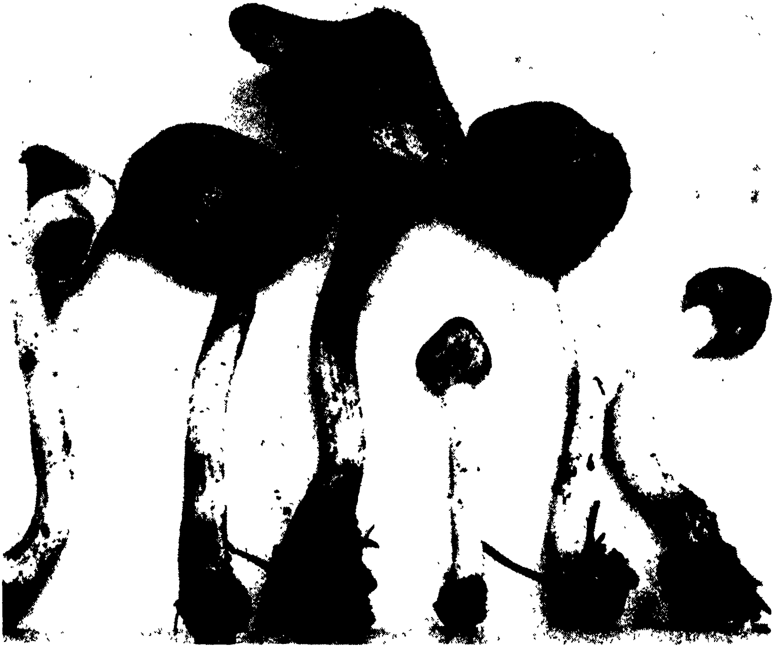

Photo D. E. Stuntz

Fig. 11. *Inocybe picrosma*, × 1.

4–5 mm., moderately close, about 90 reaching the stipe, as many more inserted, color at first pallid (parchment 12B3, cream 9D2, "ivory yellow"), becoming rather pale grayish olivaceous brown (tanaura 12D4, 12F5, prairie 13F6); stipe 2.5–8.5 cm long, 3–7 mm. thick, terete, equal, the base almost always with a narrow flat bulb which in some specimens may be emarginate, solid, context white or pallid, unchanging on exposure, but slowly in age becoming suffused all over with "avellaneous" or dull vinaceous brown or fuscous brown, surface satiny, shining, conspicuously longitudinally hygrophanous-grammate, entirely white-pruinate at first, remaining so at apex, becoming almost glabrous below, color very pale yellow ("cartridge buff," "ivory yellow," 9D1), in age becoming flushed first at the apex then all over with dull vinaceous brown ("avellaneous," "wood brown," cork 12B7, India spice 13B8) or fuscous brown ("drab," "cinnamon drab," winter leaf 15A8), the margin of the bulb almost always with a flush of "apricot buff," "ochraceous salmon" or "light ochraceous salmon."

Spores 7–8.5–10 (11) × 5–6 μ, smooth, inequilaterally amygdaliform; pleurocystidia 36–50 (63) × 16.5–21 μ, fusiform-clavate, with obtuse apex and slender pedicel, very thick-walled above; cheilocystidia of two kinds, some similar to the pleurocystidia in size and shape, others clavate, thin-walled, 22–56 × 8–11.5 μ.

Habit, habitat and distribution: Under conifers, lower Tahoma Creek, Smith, September 14 (St. *4536*); same locality, September 18 (St. *4647*); Tahoma Creek Camp Grounds, Smith, September 20 (St. *4660*); lower Tahoma Creek, Smith and Stuntz, September 21 (St. *4700*); same locality, September 23 (St. *4736*); lower slopes of Tumtum Mountain, near Tahoma Creek, Smith and Stuntz (St. *4839*—**type**).

Discussion: This *Inocybe* can be recognized quite readily by its silky pale yellow pileus, pallid yellow stipe which turns dull vinaceous brown in age, and especially by its unique odor. In coloration and general appearance it resembles *Inocybe Kauffmanii* Smith, and indeed the two species appear to be rather closely related; however, the stipe of *I. Kauffmanii* does not become vinaceous brown with age, and its odor is entirely different. There is also a superficial resemblance between *I. **picrosma*** and *I. **suaveolens*** Stuntz, but no real relationship whatever, as the two have entirely different microscopic characteristics as well as very different odors. The odor of *I. **picrosma*** is its chief distinguishing characteristic; though not actually strong, it has a peculiar pungency that sets it apart from most fungus odors and usually produces an effect similar to slight traces of sulphur dioxide. The brittleness and darkening with age characteristic of the stipe context are well marked, and seemed constant in the various collections. The stipe context is so brittle that it is difficult to collect a specimen without breaking off either the base or the pileus. The stipe of older specimens is apt to become gray in drying, like that of *Inocybe xanthomelas* Kühner.

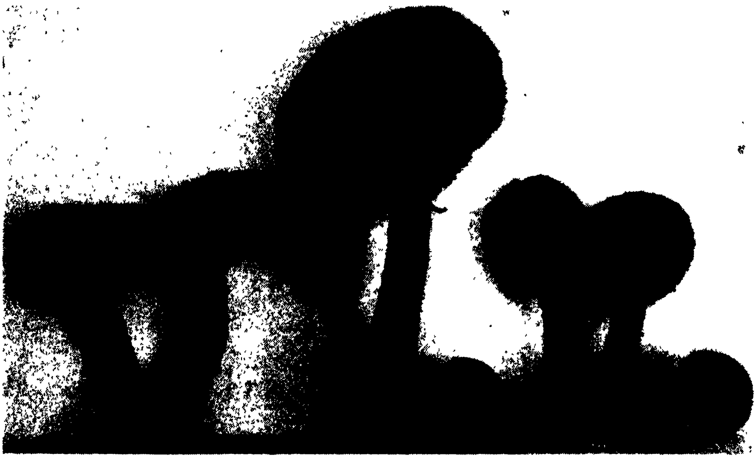

Photo D. E. Stuntz

FIG. 12. *Inocybe pyrotricha.*

Inocybe pyrotricha Stuntz, sp. nov. *Figs.* 10*e, f;* 12

Pileus 1.5–3 cm. latus, campanulatus demum expansus, late umbonatus, siccus, mox squamulosus, centro ferrugineus vel rufus, margine pallescens, sordide brunneus; caro pallida, immutabilis, odor tenuiter raphaninus; lamellae adnatae, ventricosae, 6 mm. latae, primo violaceae demum pallide brunneae; stipes 2.5–5 cm. longus, 2.5–5 mm. crassus, aequalis, solidus, sursum subglaber, pallide violaceus, deorsum fibrillis rufis dense obtectus; sporae 7–10 × 4.5–5 μ, leves, amygdaliformes; pleurocystidia 66–80 × 16.5 μ, ventricosa, pedicellata.

Pileus 1.5–3 cm. broad, campanulate, becoming campanulate-expanded with a large broadly rounded umbo, the margin entire, at first incurved, becoming gently rounded, finally almost plane; surface dry, densely appressed-fibrillose, smooth only in the youngest buds, soon diffracted scaly with small flat frequently recurved squamules at least in the center, squamulose almost to the margin in some, in others the marginal area merely fibrillose-lacerate and more or less rimose, decorated at first with the remains of the avellaneous cortina, soon denuded, color of young buds uniformly ferruginous or rufous, of older pilei "burnt sienna" to "Kaiser brown," "ferruginous" or "cinnamon rufous" at the center, some shade of dingy brown such as cork 12B7, 13E9, Mosul 14F8 toward the margin, on drying becoming generally somewhat paler, "ochraceous tawny" to "tawny" at the center, toast 13F8 at the margin, the surface fibrils finally assuming a decidedly yellow-orange cast as they dry out; context thin, firm, at first with a pale lavender tinge, soon pallid, unchanging on exposure; odor rather pleasant, subraphanoid or faintly spermatic, taste mild, a little nutty; lamellae adnate, deeply and broadly rounded at stipe and margin, symmetrically ventricose, rather broad, up to 6 mm., moderately close, about 50 reaching the stipe, with another series inserted in various lengths, and occasional marginal lamellae, color at first "pale bluish lavender" to "pale verbena violet" or "lavender" becoming "light cinnamon drab," finally adobe 14D7; stipe 2.5–5 cm. long, 2.5–5 mm. thick, terete, equal, the base a little clavate-incrassated and often abruptly truncate, solid, the context pallid within, yellow at the surface toward the base, persistently pale lavender or bluish lavender at the surface toward the apex, unchanging on exposure, surface densely peronate with a floccose-fibrillose sheath which persists on the basal two thirds, there becoming pulled, as the stipe expands, into conspicuous longitudinal streaks and reticulations which diminish in abundance upwards, the apex subglabrous, finely pruinate and minutely fibrillose, ground color at the base "Naples yellow" to "Colonial buff," at the apex "pale bluish lavender" to "lavender," the median part pallid, color of the peronate surface fibrils "Sanford's brown" to "burnt sienna" or "orange rufous" at the base, shading upward through "cinnamon rufous" to "avellaneous" near the apex, in young specimens the base is usually "zinc orange" to "ochraceous orange."

Spores 7–10 × 4–5–6 μ, usually 8–9 × 5.5 μ, with broadly rounded ventral profile; pleurocystidia 66–80 × 13.5–16.5 μ, slender, ventricose with long neck and well-defined pedicel, thick-walled; cheilocystidia of two kinds, one similar in size and shape to the pleurocystidia, the other thin-walled, clavate, 20–38 × 11–26 μ, in dense clusters, both kinds with pale brown wall.

Habit, habitat and distribution: Under conifers, base of Rampart Ridge near Longmire, September 15, collected by Smith (St. *4545* —**type**).

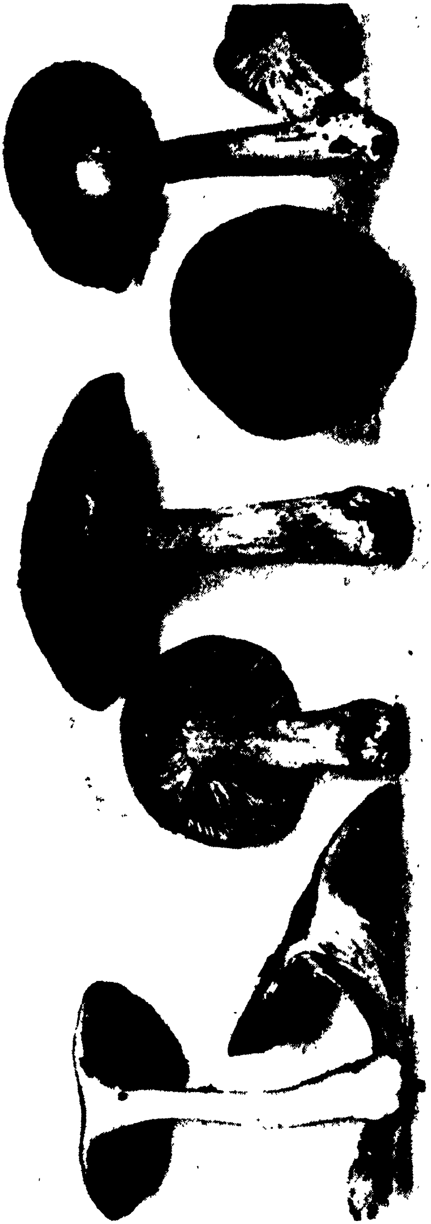

Photo D. E. Stuntz

FIG. 13. *Inocybe rainierensis*, × 1.

Discussion: This is certainly one of the most brightly colored and distinctive of the group of species closely related to *Inocybe obscura* (Fr.) Gill. The coating of rusty red fibrils on the stipe and the decidedly reddish color of the pileus surface are the features which readily distinguish it from the other members of its stirps.

Inocybe rainierensis Stuntz, sp. nov. *Figs.* 10*g, h;* 13

Pileus 1.5–4.5 cm. latus, obtuse campanulatus demum expansus, late umbonatus vel non, siccus, sericeus, centro velamine pallide brunneo obtectus, cutis obscure brunneo; caro firma, pallida, immutabilis: odor nullus vel vix raphaneus; lamellae adnatae, subconfertae, ventricosae, 4.5–7 mm. latae, ex pallidis brunneo-olivaceae; stipes 2–4.5 cm. longus, 3.5–8 mm. crassus, plerumque compressus, aequalis, basis bulbosus, sericeo-nitens, longitudinaliter fibrilloso-striatus, sursum cinnamomeo-incarnatus, deosorum brunneus; sporae 10–15 × 5–6.5 μ, crasse 4–5-tuberculatae, apice praelongae; pleurocystidia 60–86 × 16–20 μ, fusoideo-ventricosa.

Pileus 1.5–4.5 cm. broad, obtusely campanulate, becoming expanded and obtusely convex to broadly umbonate, margin persistently broadly rounded; surface dry, smooth, covered at the center with a thin, webby, appressed, persistent pallid brown coat of fibrils, true cuticle appressed-fibrillose, silky smooth, finally becoming more or less obscurely areolate to subscaly at the center, margin remaining smooth or in some finally a little rimulose, usually decorated with the rather copious pallid veil, color dark brown with an obscure reddish cast ("Verona brown," "snuff brown," Montella 8J11, 8J12, or "bister" to café noir 8H12 at center, elsewhere brownstone 7C10, Vandyk brown 7A11, dark beaver 15A9, teakwood 15C9, English oak 15A10), appearing more pallid brown (adobe 14D7) here and there because of the coating of surface fibrils; context 2–3 mm. thick off the disc, firm, pallid or tinged with brown, unchanging on exposure, odor none or faintly raphanoid; lamellae narrowly to broadly adnate, sinuate to emarginate, more or less deeply rounded at the stipe, bluntly pointed at the margin, ventricose, 4.5–7 mm. broad, moderately close, 55–65 reaching the stipe, the same number inserted in irregular lengths, color pallid at first (putty 11B2, becoming Malacca 12C4), finally olivaceous brown (airdale 14F6, 14G6, 14G7); stipe 2–4.5 cm. long, 3.5–8 mm. thick, terete or more often markedly compressed, equal above the base which has an abrupt to napiform bulb 7–11 mm. in diameter, solid, unchanging on exposure, surface densely longitudinally silky fibrillose at first, becoming longitudinally fibrillose-streaked and hygrophanous-grammate on a satiny shining surface, apex pruinate, margin of bulb densely beset with thick matted fibrillose tufts of the copious veil, color at first uniformly "light pinkish cinnamon," darkening and finally becoming brown below, at length with the apex pallid, shading down through suntan 13B7, Tuscan tan 13C8, marron glace 14A8, and finally "Verona brown," with the base eventually "warm sepia," but all these colors appearing paler because of the pallid surface fibrils.

Spores (10) 11.5–13 (16) × 5.5–9 μ, quite irregular in outline, but generally with 4 or 5 coarse nodules at the base, and the apex prolonged into a bullet-shaped structure; pleurocystidia 58–70 (78) × (12) 16–25 μ fusiform-ventricose, only moderately thick-walled; cheilocystidia same size and shape as the pleurocystidia, accompanied by a few small and inconspicuous clavate ones.

Habit, habitat and distribution: Under conifers, elevation 4800 ft., Eagle Peak, Smith, August 26 (St. *4161*); under Abies, Reflection Lake, Smith, August 28 (St. *4200*—**type**).

Discussion: This *Inocybe* and *Inocybe chelanensis* Stuntz have practically the same microscopic characters, but differ markedly in outward appearance. The pileus of *I. rainierensis* is much darker and more uniformly colored than that of *I. chelanensis,* and the stipe always has a conspicuous bulb, whereas the stipe of *I. chelanensis* lacks a bulb altogether. Unfortunately, *I. chelanensis* seems to be a rare species, as yet known only from the type collection, so that nothing can be said concerning the extent of its variability with respect to the above characteristics. In view of this situation, and since there is actually such a marked difference in the appearance of the two fungi, it seems best to consider them separate though closely related species.

Inocybe suaveolens Stuntz, sp. nov. *Figs.* 10*i, j;* 14

Pileus 2–4.5 cm. latus, campanulato-expansus, siccus, squamosus, centro albidus, margine primo albidus deinde pallide luteus vel cremeus; caro alba vel pallida, immutabilis, odor fragrans; lamellae adnexae, ventricosae, 4–5 mm. latae, ex albidae brunneo-olivaceae; stipes 3.5–8.5 cm. longus, 2.5–7 mm. crassus, aequalis, basis marginato-bulbosus, solidus, caro pallide luteola, immutabilis, longitudinaliter striatus, omnino albido-pruinatus, pallide luteus, tarde sordide brunneus vel subincarnatus; sporae 7–9 (10)·×5.5–6.5–7.5 μ, nodulosae vel subnodulosae; pleurocystidia 43–58 (60) × 10–16.5 μ, fusoidea vel sublanceolata, muro valde crasso praedita.

Pileus 2–4.5 cm. broad, campanulate, becoming expanded and broadly umbonate, margin involute then gently rounded, finally almost plane, surface dry, silky-smooth and subshining at first

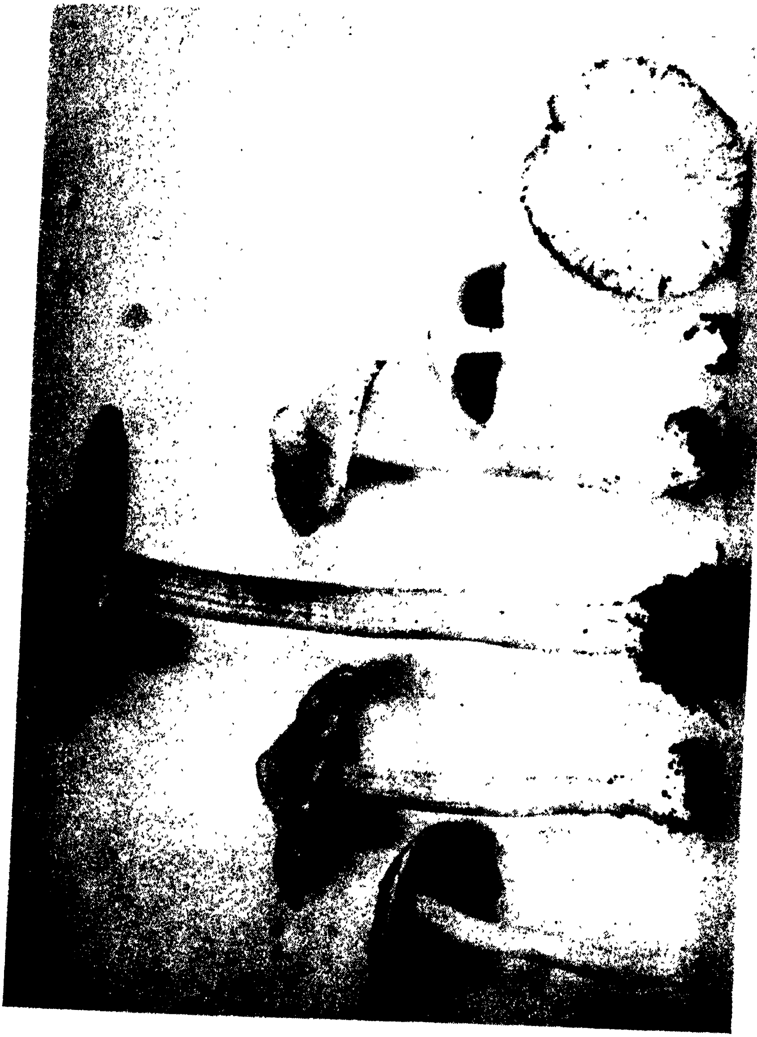

FIG. 14. *Inocybe suaveolens*, × 1.

Photo D. E. Stuntz

(lubricous when thoroughly wet), soon becoming more or less diffracted scaly, the scales with free ends, or even more or less conspicuously revolute, margin often rimose, sometimes becoming excoriate or deeply split in age, color at first uniformly white, remaining so at the center, toward the margin becoming more or less yellowish or tinged with buff ("cartridge buff" to "cream buff," or maple 11E4 or chamois 11I5); context 2–3 mm. thick off the disc, hard, white or pallid, unchanging on exposure, odor spermatic for an instant when the context is first cut open, then immediately becoming and long remaining very fragrant, almost exactly like a mixture of the odors of sweet pea and *Convallaria majalis*; lamellae adnexed, in some almost free, slightly to broadly and deeply rounded close to the stipe, bluntly pointed at the cap margin, ventricose, moderately broad (4–5 mm.), rather close, 60–70 reaching the stipe, with 2 or 3 series inserted in varying lengths, color at first pallid, becoming India buff 12E5, finally brownish olivaceous (Isabella 13K7 to maple sugar 14J8, bronze 14L9, or 14G7); stipe 3.5–8.5 cm. long, 2.5–7 mm. thick, terete or somewhat compressed, frequently flexuous, base with a distinct and sometimes conspicuous marginate bulb 6–12 mm. thick, solid, the context hard, often spirally twisted, white to pale yellowish, unchanging on exposure but becoming a little flushed with brownish or incarnate at the surface in age, surface somewhat shining, everywhere densely white-pruinate, conspicuously and broadly longitudinally hygrophanous-grammate, color at first "cartridge buff" becoming "ivory yellow" to chamois 11I5, then in age more or less flushed with brownish or incarnate shades, such as "cinnamon buff," Pablo 12G7, or even Centennial brown 13K8.

Spores 7–9 (10) × 5.5–6.5 (7) μ, merely angular to decidedly nodulose; pleurocystidia 43–58 (60) × 10–16.5 μ, subfusiform to sublanceolate, apedicellate, very thick-walled; cheilocystidia of two kinds, one like the pleurocystidia in size and shape, the other smaller, clavate, thin-walled, 15–22.5 × 9–11 μ, in dense clusters.

Habit, habitat and distribution: Under conifers, lower Tahoma Creek, Smith and Stuntz, September 18 (St. *4646*—**type**); lower slopes of Tumtum Mountain, along lower Tahoma Creek, September 26 (St. *4800*).

Discussion: The white silky pileus which becomes scaly and more or less flushed with yellow, the pruinate, marginate-bulbed, pale yellow stipe, and especially the persistent, sweet odor, closely resembling that of the common sweet pea, are the distinguishing characteristics of this species. There is no tendency of any part of the pileus or stipe context to become reddish on exposure to air, as is usually the case with Inocybes having an aromatic odor. The most closely related species appear to be *Inocybe Bresadolae* Massee sensu Kühner (**1932**, p. 158) and *I. capucina* Fr. sensu Patouillard as described by Heim (**1931**, p. 290). From both of these, *I. suaveolens* differs in the much paler color of its pileus, and the complete lack of any change to red in the exposed context. To judge from the descriptions cited above, the odor of *I. suaveolens* is also different, but probably not too much emphasis should be placed on a characteristic as difficult to judge objectively as odor.

Mycena fallax Smith sp. nov. *Fig. 3e, f*

Pileus 2–5 mm. latus, obtusus demum convexus vel planus, undulatus et plicato-striatus, glaber, udus, aquose albidus deinde candidus, membranaceus; lamellae angustae, subdistantes, praedecurrentes, albidae; stipes 0.5–1 cm. longus, filiformis, glaber, albidus, insiticius; sporae 7–8.4 × 3–4 μ anguste ellipsoideae, amyloideae; pleurocystidia fusoide ventricosa, apicibus echinulatis, 40–60 × 9–13 μ; cheilocystidia pleurocystidia similis.

Pileus 2–5 mm. broad, obtuse becoming convex, expanding to broadly convex or plane, the margin connivent to stipe at first, surface undulating and plicate-striate, glabrous and moist, watery-white or shining-white over margin and watery-white on disc, shining white over all when faded; flesh membranous and fragile; lamellae narrow, subdistant to distant, long-decurrent on the enlarged apex of the stipe, white like pileus, edges even; stipe 0.5–1 cm. long, filiform or about 0.25 mm. thick, hyaline-white and perfectly naked (no pruinosity on youngest buttons), base inserted on the substratum.

Spores 7–8.4 × 3–4 μ, narrowly ellipsoid, smooth, distinctly amyloid when fresh, weakly amyloid after standing in herbarium two years; basidia four-spored, 26–30 × 7–8 μ; pleurocystidia 40–60 × 9–13 μ, fusoid-ventricose, the apices simple to forked and with scattered echinulations, thin-walled and hyaline in KOH; cheilocystidia similar to pleurocystidia; cuticle of pileus of narrow filaments 2–3.5 μ in diam. and sparsely covered with short rod-like projections; caulocystidia present only near the gills and similar to pleurocystidia.

Habit, habitat and distribution: Gregarious on *Rubus* canes in very wet localities, Sandy, Oregon, November 5, 1947 (Sm. *28505* —**type**). Two fruiting bodies were found in the Park in a dense growth of *Rubus* near a beaver pond along lower Tahoma Creek late in September, 1948, but the material was used up in the process of identification.

Discussion: This is an easily recognized *Mycena* when fresh because of the echinulate cystidia and because the spores give a stronger amyloid reaction at that time than after the specimens have been dried. The cystidia have coarser echinulations than do those of *M. borealis* Smith. *M. fallax* seems to resemble *M. litoralis* Smith in many characters but has more strongly decurrent gills and echinulate cystidia.

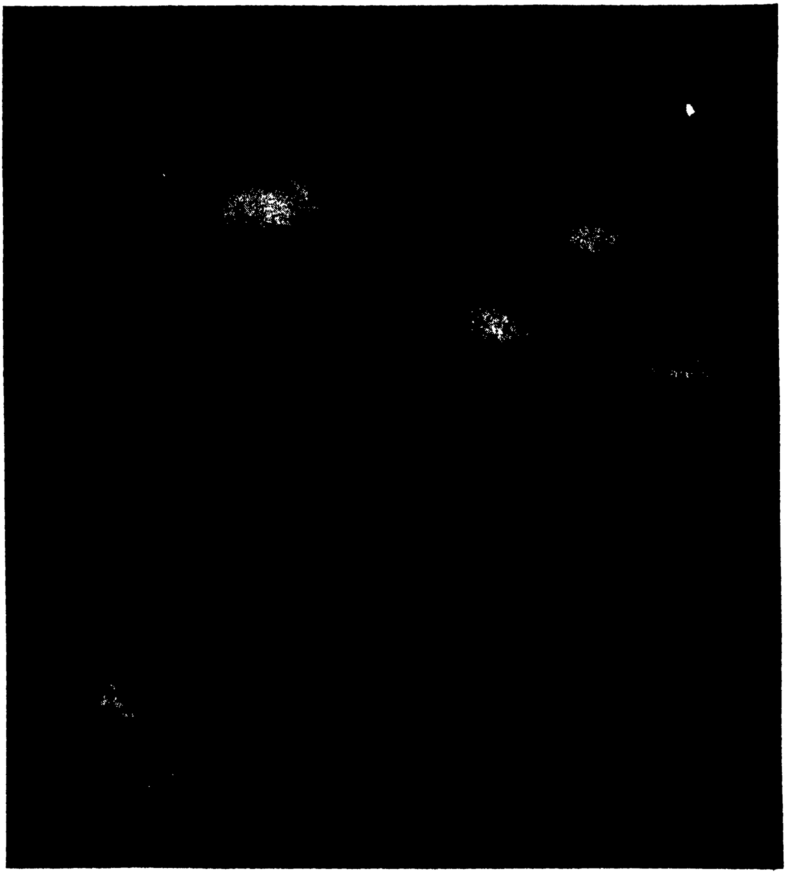

Photo A. H. Smith

FIG. 15. *Mycena rainierensis*, × 1.

Mycena rainierensis Smith sp. nov. *Figs. 3b, d;* 15

Pileus 15–30 mm. latus, convexus demum late convexus, glaber, lubricus, striatulatus, margine albidis, disco pallide griseis; lamellae albidae, latae, late adnatae, confertae vel subdistantes; stipes 5–10 cm. longus, 1.5–3 mm. crassus, aequalis, glaber, albidus; sporae 8–10 µ, punctatae; cheilocystidia 60–120 × 9–14 µ, fusoide ventricosa, apicibus acutis.

Pileus 15–30 mm. broad, convex with a straight to connivent margin, broadly convex in age, surface lubricous when wet, with fine translucent striations nearly to disc, white with a watery gray more or less sharply defined discal spot, some merely watery white to watery grayish over disc; flesh thin, pliant, odor and taste not distinctive; lamellae white, broad, bluntly adnate and horizontal, only moderately close, slightly avellaneous in age when water-soaked, edges even and concolorous; stipe 5–10 cm. long, 1.5–3 mm. diam., equal, glabrous, naked, dull white but shining when wet, transversely translucent-striate, with a slight pruinosity at line of gill attachment.

Spores globose to subglobose, hyaline, 8–10 µ in diam., amyloid, with a very thin amyloid exospore which soon disappears, endospore thicker and perforated with innumerable pores, the pore walls usually slightly amyloid; basidia two-spored and four-spored, 27–32 × 8–9 µ; pleurocystidia none; cheilocystidia 60–120 × 9–14 µ, more or less fusoid-ventricose with subacute to obtuse apices, walls thin and often flexuous, hyaline; gill trama parallel or nearly so, the cells 10–15 µ in diam., cylindric and straight or curved; pileus trama homogeneous, the surface layer of hyphae slightly gelatinous.

Habit, habitat and distribution: Gregarious in a wet area along a stream, Longmire Camp Ground, September 27 (Sm. *31845*—**type**).

Discussion: Only the one collection was found. This species is obviously closely related to *M. bisphaerigera* (Lange) Smith by its spores and gill characters, but differs sharply in having the greatly elongated cheilocystidia as well as paler color. The latter character alone, however, would not be regarded as very significant. *Mycena cineraria* Smith differs in having ellipsoid spores, dark colors, and pleurocystidia. Both the pleuro- and cheilocystidia of *M. cineraria* differ in shape from the cheilocystidia of *M. rainierensis.*

Favre (**1948**) has published a very interesting account of the variants of *Mycena* (*Fayodia*) *bisphaerigera* He describes

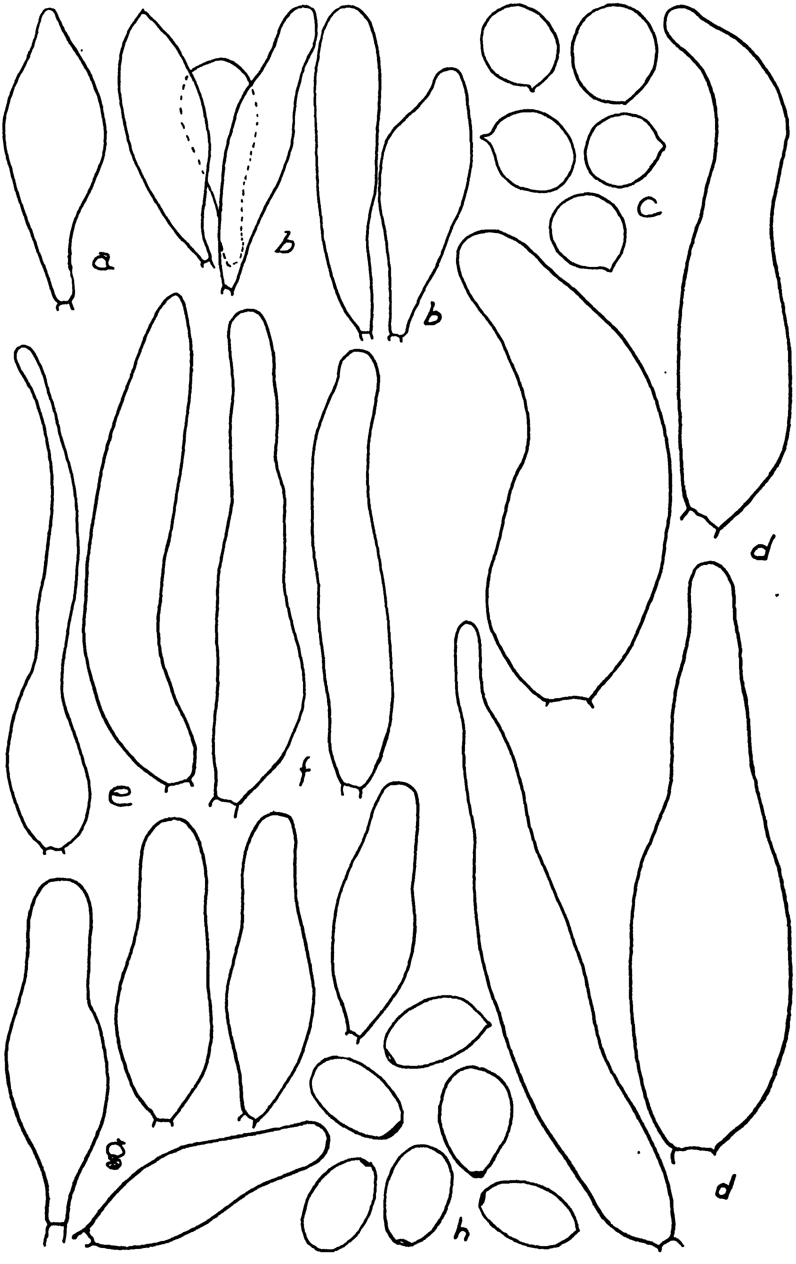

FIG. 16. Spores and cystidia of *Omphalina* and *Psathyrella*.

Fayodia bisphaerigera var. *anthracobia* as a new variety having cheilocystidia like those of *M. rainierensis*, but with the cap very dark in color and the stipe very short (18 × 1.5 mm.). Its spores measure 6–8.5 μ in diam. His variety *longicystis* has more the stature of *rainierensis* but differs in the greatly elongated cystidia. Without question Favre's two varieties and *M. rainierensis* should all be treated as of equal rank. I prefer to regard them as species, and hence describe *M. rainierensis* at that level. This procedure more closely maintains the established concepts of species in *Mycena,* and is certainly of practical value if *Fayodia* is recognized as a genus. As Favre himself pointed out, his varieties differ from each other more sharply than do many related species in other genera, such as *Clitocybe.*

Omphalina isabellina sp. nov. *Fig.* 16*c, d, e, f*

Pileus 5–15 mm. latus, convexus, demum late convexus, impolitus demum subfurfuraceus, hygrophanus, olivaceus vel sub-olivaceus deinde Isabellinus vel subochraceus, striatus; lamellae decurrentes, distantes, latae, olivaceae vel subolivaceae; stipes 1.5–3 cm. longus, 1–2 mm. crassus, aequalis, subolivaceus vel olivaceo-luteus, pubescens demum glaber vel impolitus; sporae 5–6.5 (7) μ, globosae, amyloideae; pleuro- et cheilocystidia nulla; pilo- et caulocystidia ventricosa vel subcylindrica, distinctissima.

Pileus 5–15 mm. broad, convex with a curved in margin when young, broadly convex to nearly plane in age, the margin finally wavy or recurved, surface moist and hygrophanous but with a velvety sheen or minutely scurfy from projecting pilocystidia, "sepia" to dark "Isabella color" or more olivaceous at first, becoming "Isabella color" to "light brownish olive," finally "olive ocher" to "honey yellow" with "light brownish olive" to "Isabella color" striations, sometimes eventually pale yellow and striations inconspicuous; flesh dark olivaceous, pliant but in age fragile, odor and taste not distinctive; lamellae decurrent, distant, broad, tapered to either margin, "deep olive buff" to pallid olivaceous yellow, usually not as yellow as cap and stipe, edges even; stipe 1.5–3 cm. long, 1–2 mm. thick, equal, concolor with cap margin to ocher yellow, more sordid downward, pubescent at first from projecting caulocystidia but in age glabrous to unpolished.

Spores globose to subglobose, 5–6.5 (7) μ, smooth, amyloid; basidia 40–50 × 9–10 μ, four-spored, hyaline in KOH; pleurocystidia and cheilocystidia none; gill trama interwoven, central core of very large cells which are pale yellow in water mounts of fresh material, merely pale yellow revived in chloral-hydrate-iodine solution and hyaline revived in KOH; pileus trama loosely interwoven, cuticle composed of a turflike covering of large pilocystidia with smoky yellow contents (H_2O mounts of fresh material), the pilocystidia arising from enlarged cells also having smoky yellow contents (when revived in KOH contents of both types of cells dull brown); pilocystidia more or less fusoid ventricose with obtuse apices, 40–70 × 9–16 μ; caulocystidia similar to pilocystidia or with greatly elongated necks above ventricose base, some subcylindric, 40–120 × 7–12 μ.

Habit, habitat and distribution: Gregarious to scattered on very old mossy Douglas fir logs, lower Tahoma Creek, August 14, Stuntz (Sm. *30087*—**type**). A previous collection of the same species was made by the senior author on a similar Douglas fir log at Rhododendron, Oregon, October 14, 1944 (Sm. *19695*).

Discussion: This species is very easily recognized by the Isabella to olive colors, velvety cap and hoary to pubescent stipe. It is most interesting, however, in view of recent attempts to revise generic concepts among the white spored agarics. This species is excluded from *Mycena* in the concept of Smith (**1947**) by having decurrent gills and an incurved cap margin. Singer (**1942**, pp. 128–9) published a short key separating the Marasmioideae with amyloid spores. *O. isabellina* has some of the characters of his *Hydropus,* but does not fit readily into any of the genera. Actually, our species does not appear to belong in Singer's Marasmioideae in spite of a faint resemblance in stature and consistency to *Xeromphalina campanella. O. isabellina* does, of course, fall readily into the Friesian genus *Omphalia* (*Omphalina* if the International Rules are followed), but would doubtless be excluded by those who restrict *Omphalina* to species with nonamyloid spores. However, we prefer to place it here at least for the present, where it can be found easily.

Psathyrella alboalutacea Smith sp. nov. *Figs.* 3*g, h;* 16*a, b;* 17

Pileus (2) 3–5 (6) cm. latus, obtusus vel convexus, demum planus vel subumbonatus, siccus, innato-fibrillosus demum squamulosus, squamulis albidis demum alutaciis; margo appendiculatus; lamellae adnatae, secedentes, confertae, angustae, latae, albidae demum subfuscae ("hair brown"); stipes 3–5 cm. longus, 10–12 mm. crassus, durus, farctus, intus albidus vel deorsum incarnatus, deorsum fibrillosus, sursum striatus; sporae 7.5–9 × 4–4.5 μ;

pleurocystidia clavata vel subvesiculosa, pedicellata, 25–40 × 10–14 μ, vel fusoidea et apicibus obtusis vel subacutis, 40–53 × 9–14 μ.

Pileus (2) 3–5 (6) cm. broad, obtuse to convex at first, becoming plane or nearly so, the disc in some elevated as a low broad umbo, surface dry and fibrillose, the fibrils innate and forming an

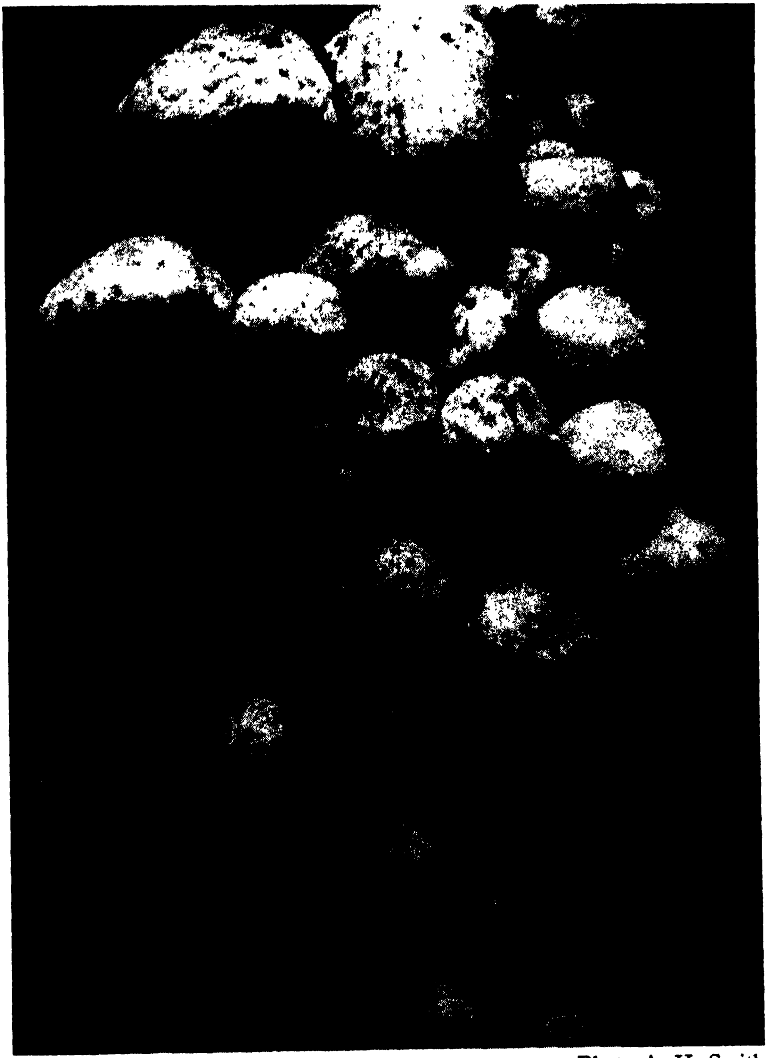

Photo A. H. Smith

FIG. 17. *Psathyrella alboalutacea*, × 1.

appressed, cottony mat over the buttons which are pure white, the fibrils soon becoming aggregated into patches and discoloring to avellaneous or wood brown, the disc at times sordid alutaceous, at maturity entire surface off-color toward cinnamon buff or disc brownish (near cinnamon brown), margin fibrillose-appendiculate to cottony-appendiculate at first but finally nearly naked; flesh firm and hard (for this genus), hygrophanous, watery avellaneous fading to whitish, odor faintly fragrant but soon fading, taste not distinctive; lamellae depressed-adnate, seceding, close, narrow to moderately broad, white to very pale avellaneous becoming drab gray to hair brown, edges white-floccose; stipe 3–5 cm. long, 10–12 mm. thick, firm and hard, stuffed but becoming hollow, white within but pinkish to orange-pink in the base, surface usually with discolored fibrils over lower half, in others white fibrillose, upper half white and longitudinally striate.

Spores 7.5–9 × 4–4.5 μ, smooth, dark cocoa-color revived in KOH, narrowly oval to suboblong in face view, in side view the ventral line slightly concave and dorsal line slightly convex, apical pore present but inconspicuous; basidia four-spored, hyaline in KOH, 28–34 × 5.5–7 μ, narrowly clavate; paraphyses basidioid; pleurocystidia rare to scattered and mostly imbedded, of two types, the first clavate to subvesiculose-pedicellate, 25–40 × 10–14 μ, thin-walled, smooth and hyaline in KOH, the second type fusoid and 40–53 × 9–14 μ, thin-walled, hyaline in KOH, smooth, apices obtuse to subacute; this type varying to submucronate, when fresh often filled with oil droplets; cheilocystidia mostly similar to fusoid type of pleurocystidia or more ovate-pointed, a few clavate to subcylindric, 32–48 × 9–14 μ, hyaline and smooth in KOH; gill trama hyaline in KOH; pileus trama with a thick (300 μ) layer of veil hyphae bearing clamps, this layer very loosely interwoven and all but surface elements (which are discolored) hyaline in KOH, beneath this a cuticle of compactly arranged cells appearing vesiculose to pseudoparenchymatic in cross section (these hyaline in KOH), tramal tissue beneath cuticle hyaline in KOH.

Habit, habitat and distribution: Cespitose on dead alder wood (stumps, logs, etc.), July 28, lower Tahoma Creek, Smith *29505* —**type.** Additional collections from the same locality are as follows: July 23 (Sm. *29308*); August 7 (Sm. *29916*); August 20 (Sm. *30360*); August 22 (Sm. *30421*); August 25 (Sm. *30591*); August 27 (Sm. *30687*).

Discussion: The distinguishing features of this fungus are the innately white fibrillose caps of button stages, the fibrils or scales

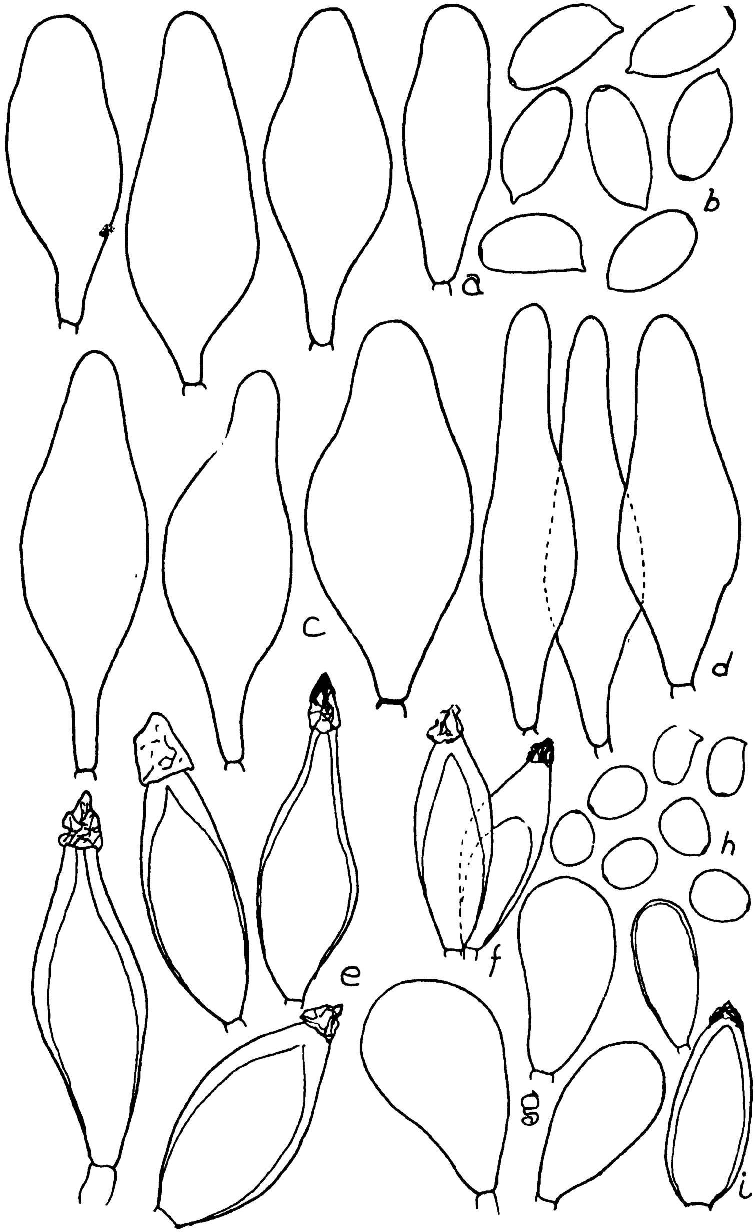

FIG. 18. Spores and cystidia of *Psathyrella*.

discoloring to alutaceous at maturity or in age, the spores (7.5–9 μ long), the two types of pleurocystidia, the pinkish to pinkish orange discoloration in the base of the stipe, and the clustered habit. It appears to be most closely related to *P. maculata*, a species described by Parker from Atkinson's Mt. Rainier collections. It differs in having larger spores and different pleurocystidia as well as in the color of the scales on the cap. In *P. maculata* the young caps are nearly fuscous. Both have been found growing on the same alder stub. It is unquestionably closely related to *Hypholoma scobinaceum* of European authors, but the gills are never tinged flesh color, and the pileus is never fuscous, unless very old discolored specimens are taken into account.

Psathyrella candidissima Smith sp. nov. *Figs.* 16*g, h;* 18*a*

Pileus 2–4 cm. latus, obtuse conicus demum subumbonatus, albo-fibrillosus demum squamulosus, glabrescens, candidus; lamellae confertae, angustae, adnatae, secedentes, candidae demum subfuscae ("light drab"); stipes 5–10 cm. longus, 4–6 mm. crassus, aequalis, cavus, candidus, floccoso-fibrillosus, subglabrescens; sporae 8–10.5 × 4–5 μ; pleurocystidia 32–40 × 10–15 μ, ventricosa, apicibus late rotundis.

Pileus 2–4 cm. broad, obtusely conic at first, expanding to nearly plane or with a low obtuse umbo, surface at first coated with a layer of snow-white fibrils more or less radially arranged and which become aggregated into fascicles before disappearing entirely, surface glabrous in age, snow-white beneath the fibrils when young, scarcely changing color in age or only the disc becoming cream color, the margin appendiculate from remains of the veil; flesh very brittle but also soft, watery pallid, fading to white, odor and taste not distinctive, no color change when bruised; lamellae close, thin, narrow, ascending-adnate and soon seceding, snow-white, becoming "light drab," edges even; stipe 5–10 cm. long, 4–6 mm. thick at apex, equal, hollow, fibrous, snow-white throughout, surface floccose-fibrillose from veil and at first with a slight evanescent zone near apex or above middle, floccose-pruinose above the zone.

Spores 8–10.5 × 4–5 μ, smooth, subelliptic in side view, in face view elliptic to subovate and broadest near base, chocolate color revived in KOH, pore apical but indistinct; basidia four-spored, hyaline in KOH, 18–23 × 7–8.5 μ; paraphyses basidioid; pleurocystidia scattered to abundant, ventricose with short broad necks and broadly rounded apices, 32–40 × 10–15 μ, smooth, thin-walled, hyaline in KOH; cheilocystidia abundant, hyaline, fusoid-ventricose, apices obtuse, 32–50 × 8–12 μ, some small inflated to clavate cells also present; gill trama hyaline in KOH, subhymenium cellular; pileus trama hyaline in KOH, cuticle of a layer of pseudoparenchymatic cells only somewhat larger than diameter of cells of the flesh.

Habit, habitat and distribution: Gregarious on alder debris, lower Tahoma Creek, about 100 yards below the old Tahoma Creek Forest Camp, August 6, Sm. *29871*—**type.**

Discussion: The outstanding features of this species are the lack of an annulus, medium sized spores and broadly rounded pleurocystidia. Although found but once the species is so outstanding that it merits description. A somewhat similar species, which occurs in the Cascades farther south, has a very fragrant odor and varies in the presence of an annulus. In some respects *P. candidissima* resembles *P. insignis* but the spore size, 6.2–7.5 × 3.2–3.8 μ as compared to 8–10 × 4–5 μ, distinguishes them at once. *P. candidissima* belongs in the subgenus *Hypholoma*. *Hypholoma cascum* as described by Fries is quite similar in many respects, but Fries did not emphasize that his plant was shining white over all, in fact he described it as "griseo-alutaceo-albicans" on pileus and the gills "e griseo nigrofuscis." He does not attribute to it either a fragrant odor or bitter taste. This is important in view of descriptions by later authors. Fries' description in Monographia (p. 426) puts even more emphasis on the color of the pileus. Consequently it does not seem justifiable to identify the Mt. Rainier fungus with his species. The difference in width of the gills as expressed in our description and in all those by Fries (narrow as contrasted to "perlatis") would appear to be an additional distinguishing character.

Psathyrella caput-Medusae (Fr.) comb. nov.* *Figs.* 18*b c, d; 19*

Pileus 4–5 cm. broad at base, nearly 3 cm. high, obtusely campanulate, surface at first covered with small whitish superficial fibrillose scales which show a tendency to become fuscous or bister at the tips, glabrescent, surface dark snuff-brown to pale snuff-brown on disc, near sepia toward the margin, hygrophanous and fading in streaks to give marginal area a coarsely striate appearance, near cinnamon buff faded; flesh firm but fragile, near snuff-brown moist, fading to near avellaneous, taste mild, odor sweetish aromatic; lamellae close to crowded, broad, ascending adnate, avellaneous becoming wood brown, edges even to slightly crenulate; stipe 8–10 cm. long, 7–8 mm. thick at apex, equal, hollow but not exceptionally fragile, cavity lined with avellaneous tissue, cortex

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* *Agaricus caput Medusae* Fries, Epicr. p. 216. 1838.

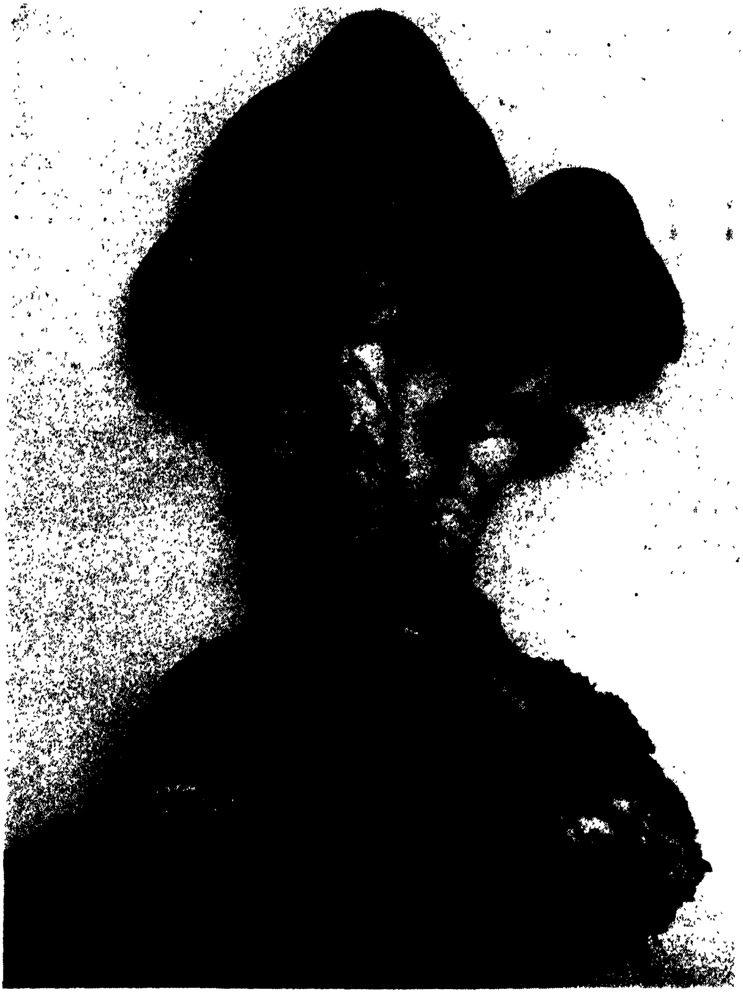

Photo A. H. Smith

FIG. 19. *Psathyrella caput-Medusae.*

paler (whitish), surface white and densely fibrillose-squarrose scaly below the cottony-membranous, fringed annulus, scales white but tips fuscescent, pruinose-silky-striate above the superior ring. Spores 9–11.5 × 4.5–6 μ, bright cocoa-color revived in KOH but darkening somewhat on standing, subinequilateral to subelliptic in side view, apiculus distinct, suprahilar depression slight, in face view broadest near the ovate-pointed base, evenly tapered to a rounded apex, apical pore not visible under oil; basidia clavate, four-spored, 23–28 × 9–10 μ, hyaline in KOH; pleurocystidia 36–54 × (12) 14–20 (22) μ, hyaline in KOH, broadly ventricose with a short neck ending in an obtuse apex, thin-walled, many with numerous minute oil droplets; cheilocystidia similar to pleurocystidia or narrower and more elongated, 50–65 × 9–13 μ, the neck often drawn out and flexuous, apices obtuse, some with oily content, some homogeneous, thin-walled and hyaline in KOH; gill trama parallel, hyaline in KOH or at first flushed cinnamon; pileus trama parallel, hyaline in KOH or soon fading (flushed cinnamon when first revived), cuticle a layer of pseudoparenchyma 3–5 cells deep, the cells not greatly enlarged.

Habit, habitat and distribution: Cespitose on a conifer stump near Reflection Lake, September 3, collected by Stuntz (Sm. *30907*).

Discussion: The diagnostic features of this species are the habitat on conifer wood, the sweetish-aromatic odor, the darkening veil remnants, obtuse pleurocystidia many of which have oily contents, spore size, and very obscure apical pore of the spore. We have a somewhat similar species in our western mountains with a white pileus and different habitat which will be considered in a later paper. According to our observations *P. caput-Medusae* is an exceedingly rare fungus in North America. We know it from only this one collection. Morgan (**1908**) recognized it on the basis of its being reported from the Pacific Coast but ascribed spores 16–18 × 5 μ to it. We have not examined specimens, and exclude this report on the basis of the description. The Friesian account (Epicr. p. 216) describes the essential features of our collection. Lange's description and illustration cover our material even better. It remains to be seen whether the oily content of some of the cystidia is a character of importance. At present we do not so regard it. It seems apparent that the species has been variously treated by European authors. Rea (**1922**) classifies it as to group in having an innately fibrillose pileus but in his description says it soon becomes smooth. He gives the spore size as 8–9 × 4 μ and describes the veil remnants as fuscous, not fuscesent. Bresadola states that it has no distinct odor, but in other respects his account covers our collection quite well. Through the courtesy of Seth Lundell, Upsala, Sweden, we have examined a collection by B. Norkrans from conifer wood near Upsala. The material was collected November 19, 1945 and identified by Lundell as *Hypholoma caput-Medusae*. Our Mt. Rainier collection is similar to this collection. In both the apiculus, obscure germ pore, and shape of the spore are identical as are also the pleuro- and cheilocystidia, thick cuticle of the pileus, and lack of color in the flesh of the pileus when the latter is revived in KOH. The species finds a logical place in *Psathyrella* among the annulate species of the subgenus *Hypholoma.*

Psathyrella Naucoria Smith sp. nov. *Fig.* 18*e, f, g, h, i*

Pileus 1–3 cm. latus, late convexus demum planus, margine incurvo, demum recurvo, glaber, hygrophanus, cinnamomeo-brunneus dein pallide argillaceus; lamellae pallidae demum cinnamomeae vel cinnamomeo-purpureae, confertae, angustae, late adnatae; stipes 1–2.5 cm. longus, 2–3 mm. crassus, glaber, cartilagineus, pallide ochroleucus demum subargillaceus; sporae in cumulo "Verona brown," 5–5.8 (6) × 3.5–4 × 4–4.7 μ, compressae; pleurocystidia 28–36 × 8–14 μ, fusoide ventricosa vel ventricosa-mucronata, crassotunicata, apicibus incrustatis.

Pileus 1–3 cm. broad, broadly convex with an incurved margin, becoming plane or margin uplifted, glabrous, when moist striatulate on margin, hygrophanous, "cinnamon brown," moist, fading to "cinnamon buff" or "pinkish buff," fading on the disc first; flesh thin but relatively firm, pallid, no odor or taste; lamellae pallid young, soon tinged cinnamon and becoming dark cinnamon-brown, finally with a purplish red tinge, close to crowded, narrow, horizontal and bluntly adnate, becoming shallowly adnexed, edges even; stipe short, 1–2.5 cm. long, 2–3 mm. thick at apex, usually curved, glabrous (no veil on buttons), stuffed with a pallid to white pith, distinctly cartilaginous-pliant rather than fragile, surface pruinose above, pallid honey-color young, cinnamon buff or darker in age, base slightly mycelioid.

Spores "Verona brown" to near "warm sepia" in deposits, very pale under the microscope in H_2O mounts of fresh material, when revived in KOH immature spores pale cocoa-gray, 5–5.8 (6) × 3.5–4 × 4–4.7 μ, at least some slightly compressed, broadly subovate to subglobose in face view, subelliptic in side view, pore apical but minute; basidia four-spored, 15–17 × 5.5–6.3 μ, hyaline in KOH; paraphyses basidioid; pleurocystidia abundant, 28–36 × 8–14 μ, fusoid-ventricose to ventricose-mucronate, thick-walled at least above and mucro usually incrusted with an exudate, hyaline in KOH; cheilocystidia similar to pleurocystidia or thin-walled and vesiculose to clavate, 26–34 × 10–16 μ, hyaline in KOH; gill trama parallel or nearly so, hyaline in KOH; pileus trama hyaline in KOH, cuticle of an irregular palisade of clavate cells from between many of which clavate to sub-cylindric pilocystidia arise, these sometimes elongated into filaments, clamp connections present at cross walls.

Habit, habitat and distribution: Gregarious to scattered on very decayed alder logs, lower Nisqually River, August 3, Sm. *29785*—**type.** Coll. Sm. *30328* was made from the same log August 18.

Discussion: This species closely resembles **Psathyrella camptopoda** (Pk.) Smith comb. nov.* in habit, habitat on hardwood logs, pale spores under microscope, thick-walled pleurocystidia and stature. It differs chiefly in its much broader (in face view) slightly compressed spores. The color of the spores in KOH clearly indicates its proper position in *Psathyrella* rather than in *Naucoria*. The purplish red tinge of the gills develops slowly so that it is not too reliable as a generic character.

Psathyrella rubicola Smith sp. nov. *Figs.* 20*a, b, c, d, e*

Pileus 2.5–4 cm. latus, convexus vel subconicus, demum planus, albofibrillosus demum fibrilloso-squamulosus, glabrescens, margine appendiculatus, hygrophanus, sordide luteo-brunneus dein pallide argillaceus; lamellae pallidae ("tilleul buff") demum pallide fuscae, confertae, latae, late adnatae, secedentes; stipes 4–5 cm. longus, 3–5 mm. crassus, cavus, fragilis, candidus, fibrillosus, glabrescens et aquose pallidus; sporae 7.5–9 × 4–4.5 μ; pleurocystidia 46–60 × 9–14 μ, acuta, in KOH pallide vinaceo-tincta.

Pileus 2.5–4 cm. broad, ovoid to convex, becoming obtusely conic to convex or finally plane, surface at first covered with white fibrils which soon are grouped into fascicles and disappear (over disc first), margin conspicuously fibrillose-appendiculate from remains of the thick, fibrillose veil, surface beneath fibrils "tawny olive" but eventually darkening to drab from the spores, margin translucent-striate in age before fading, fading to near cinnamon buff or paler; flesh watery, very fragile, no odor; lamellae "tilleul buff" becoming "wood brown" to paler fuscous, close, broad, broadly adnate, readily seceding, edges even; stipe 4–5 cm. long,

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* *Agaricus camptopus* Peck, Ann. Rep. N. Y. State Mus. 53: 845. 1900.

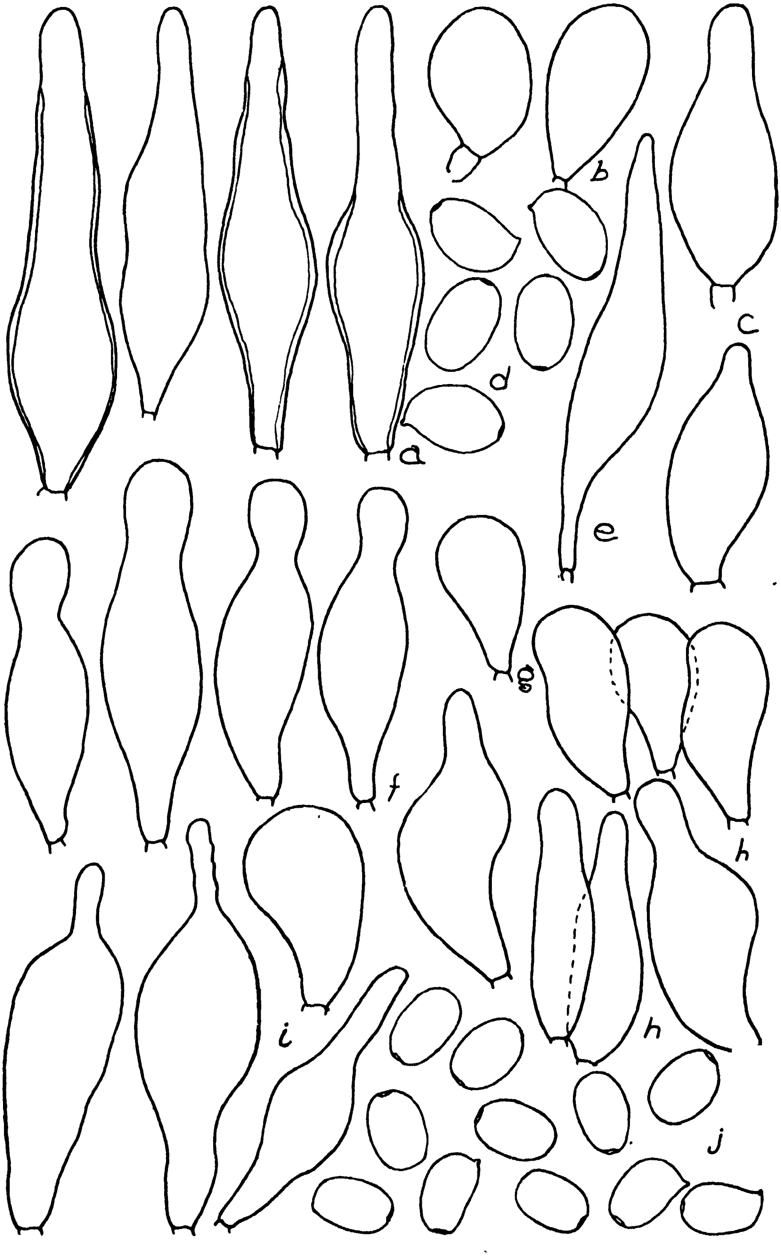

FIG. 20. Spores and cystidia of *Psathyrella.*

3–5 mm. at apex, enlarged slightly downward, hollow, very fragile, white at first because of the dense fibrillose covering, usually with a superior fibrillose zone from broken veil, also fibrillose-floccose above the zone, watery pallid to grayish beneath the fibrils.

Spores 7.5–9 × 4–4.5 μ, ellipsoid, smooth, terete, chocolate color revived in KOH, apical pore small but distinct; basidia hyaline in KOH, four-spored, 20–24 × 8–9 μ; paraphyses basidioid; pleurocystidia 46–60 × 9–14 μ, very abundant, fusoid-ventricose with acute apices, as revived in KOH with slightly thickened walls and hyaline to faintly vinaceous in KOH, the walls often flexuous, apices smooth; cheilocystidia of two types, clavate to vesiculose and up to 12–14 μ broad, these thin-walled and hyaline or faintly yellowish only at the base, second type fusoid-ventricose with acute apices, 28–40 × 9–15 μ, thin-walled, smooth and hyaline in KOH; gill trama pale cinnamon and soon fading to hyaline in KOH; pileus trama in KOH tinged cocoa-brown at first, becoming bright rusty brown just under the cuticle and paler elsewhere after standing a few minutes, cuticle a layer of vesiculose cells several cells deep.

Habit, habitat and distribution: Single or in groups of 2–3 carpophores on decaying canes and roots of Rubus species (occasionally along rotten alder logs, but here the possibility of a connection to Rubus could not be ruled out), lower Tahoma Creek about a mile above the old Tahoma Creek Camp Ground, July 19 (Sm. *29142*—**type**). Additional collections are Sm. *29148* (1 carpophore) and Imshaug *2070* (1 carpophore).

Discussion: This species is distinguished from all other species of *Psathyrella* known to me by the combination of spore size (7.5–9 × 4–4.5 μ) and acute pleurocystidia which as revived in KOH have slightly thickened walls and are frequently tinged vinaceous. There are at least two other species in North America with pleurocystidia similar to those of *P. rubicola* but both have distinctly smaller spores. They will be discussed in a future paper. Macroscopically the heavy, fibrillose, white veil and habitat are distinctive. I suspect the species of being typically vernal in its seasonal fruiting habits as it was apparently near the end of its fruiting cycle when first discovered. The species belongs in the subgenus *Hypholoma*.

Psathyrella subalpina sp. nov. *Fig.* 20*f, g, h, j*

Pileus 2–4 cm. latus, obtusus demum late convexus vel planus, saepe subumbonatus, canescens, glabrescens, hygrophanus, subspadiceus demum subcinnamomeus; lamellae confertae, adnatae, angustae demum latae, avellaneae demum purpureo-cinnamomeae; stipes 3–6 cm. longus, 4–6 mm. crassus, sursum attenuatus deorsum pallide fibrillosus, sursum pruinosis, brunneus, deorsum fuscescens; sporae 6–7 × 4–4.5 μ; pleurocystidia 34–46 × 8–12 μ, ventricosa, apicibus subcapitatis vel obtusis.

Pileus 2–4 cm. broad (probably larger when fully mature), buttons obtuse and expanding to convex or plane, occasionally with a broad low umbo, surface at first hoary to near center from a thin coating of veil fibrils, glabrescent over all except the incurved margin which retains a zone of avellaneous fibrils, surface moist and hygrophanous, "russet" at first but shading to cinnamon in fading; flesh thick, watery cinnamon-brown, taste none, odor imperceptible or very faint and reminding one of cinnamon; lamellae close, adnate, narrow to moderately broad, near avellaneous in buttons, changing through wood-brown to a dark purplish-cinnamon-brown, edges even; stipe (2) 3–6 cm. long, (3) 4–6 mm. diam., somewhat enlarged downward and with a mycelioid base sunken into the soil, surface pallid-fibrillose from the pale avellaneous veil, pallid and pruinose near apex, hollow, brownish within and cinnamon-brown in the base, lower half glabrescent, in old carpophores darkening from base upward.

Spores 6–7 × 4–4.5 μ, chocolate-color revived in KOH, smooth, very slightly bean-shaped in side view, in face view oblong to obscurely truncate-oblong, apical pore distinct; basidia four-spored, 20–24 × 6–7.5 μ, hymenium in thin sections hyaline, tinged cinnamon-yellowish in thick sections; paraphyses basidioid; pleurocystidia abundant, thin-walled, hyaline and smooth in KOH, 34–46 × 8–12 μ, ventricose with a narrowed neck and subcapitate apex but apex typically obtuse rather than broadly rounded; cheilocystidia of two types, the first similar to pleurocystidia, the second clavate to subsaccate and 12–18 × 7–12 μ, thin-walled, hyaline; gill trama parallel or nearly so, tinged cinnamon when revived in KOH, but soon fading, hyaline in mounts of fresh material in H_2O and KOH; pileus trama dull cinnamon revived in KOH, hyaline or nearly so in fresh material as mounted in H_2O, brownish in KOH, cuticle a layer of elongated to vesiculose cells arranged in more or less upright chains, the vesiculose cells usually the two rows nearest the surface, yellow in water mounts of fresh material, cinnamon in KOH, when revived in KOH dark cinnamon brown to russet.

Habit, habitat and distribution: Scattered on soil forming face of bank along trail, roots of conifers close by, elev. ± 4500 ft., September 10, collected by D. E. Stuntz (Sm. *31105*—**type**). Additional collections are: Sm. *30166*, August 15, and Sm. *30646*, August 26, all from the same spot as the type.

Discussion: The small spores and avellaneous veil distinguish this species, but the most interesting character is the structure of the cuticle of the pileus. In nearly all members of the subgenus the cells of this layer are hyaline either in water or revived in KOH, but in this species they are colored and when revived in KOH become very dark rusty brown much as in the cells forming the surface layer of the pileus in *Cystoderma amianthinum*. Although no globose cells become dusted over the pileus in *P. subalpina*, the manner in which the cuticle forms is quite similar in both species. If, in *P. subalpina*, the hyphae which produce the cuticular cells underwent a larger number of divisions and the re-

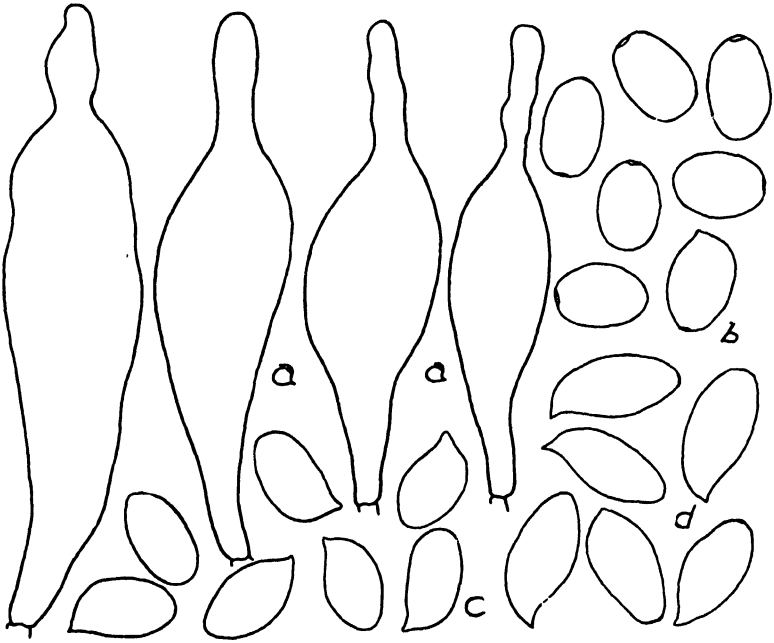

FIG. 21. Spores and cystidia of *Psathyrella* and *Clitocybe*.

sulting chains of cells broke up at least in their terminal portions into individual cells, we would have the cystoderma-type of cap covering. This is of interest to students of phylogeny within the Coprinaceae because of a large number of species in *Coprinus* and a few in *Pseudocoprinus* which do have granulose pilei—the granules supposedly derived in a large part from the tissue of the universal veil.

Psathyrella subtenacipes Smith sp. nov. *Figs. 20i; 21a, b*

Pileus 1–2.5 cm. latus, obtuse conicus demum subumbonatus vel planus, udus, hygrophanus, pallide cinnamomeo-brunneus deinde pallide argillaceus, margine appendiculatus; lamellae confertae vel subdistantes, late adnatae, latae, pallidae demum subfuscae; stipes 3–4.5 cm. longus, 3–3.5 mm. crassus, deorsum subincrassatus, cavus, subtenax, albidus, fibrillosus; sporae 7.8–9.3 × 5–5.6 µ; pleurocystidia *P. delineatae* similis.

Pileus 1–2.5 cm. broad, obtusely conic, expanding to plane or slightly umbonate, surface moist and hygrophanous beneath a coating of white universal veil fibrils so thin it does not obscure the ground color, ground color pale cinnamon brown to clay-color moist, fading to cinnamon-buff on the disc and pinkish buff elsewhere, glabrescent or remaining slightly silky, margin appendiculate at first; flesh very thin and fragile, watery brownish fading to nearly pallid, odor none; lamellae close to nearly subdistant, bluntly adnate, moderately broad, pallid young, near hair brown in age; stipe 3–4.5 cm. long, 3–3.5 mm. at apex, slightly and evenly enlarged downward, hollow, cortex pallid and fibrous, *not distinctly fragile for this genus,* white but dull, more or less evenly appressed fibrillose from the remains of the veil (no zones or scales).

Spores 7.8–9.3 × 5–5.6 µ, chocolate-color revived in KOH, smooth, elliptic to subelliptic in side view, pore apical and very small, in face view elliptic to ovoid; basidia 26–30 × 7–8.4 µ, narrowly clavate, four-spored, hyaline in KOH, paraphyses basidioid; pleurocystidia 50–70 × 10–15 µ, abundant, ventricose-mucronate but mucro elongating into a flexuous almost filamentose tip with an obtuse apex, ventricose portion usually widest above the middle and tapered to the base which arises in the subhymenium or gill trama, with a large oil drop when fresh material is mounted in H_2O but homogeneous and hyaline in KOH, smooth as revived in KOH but apex with amorphous material incrusted on it as seen in water mounts of fresh material; cheilocystidia similar to pleurocystidia or smaller, some clavate to vesiculose cells present; gill trama hyaline in KOH; pileus trama hyaline in KOH, the cuticle of clavate to vesiculose cells 2–3 cells deep.

Habit, habitat and distribution: Single to gregarious on debris among willows and cottonwood, lower Nisqually River above junction with Kautz Creek, August 3, Sm. *29757*—**type.** Only the one collection preserved.

Discussion: Single specimens of this species were encountered and found not to "run down" in my key. Since most were old it was assumed that the cystidia were abnormal. When young and freshly matured specimens were finally obtained it was realized that the species was one not previously encountered. Unfortunately, after that no more material was found. The shape of the cystidia places this species beside *P. delineata*, but there is little resemblance between the two in general appearance. A rather good field character, for any one well acquainted with the fragile nature of nearly all the smaller species of *Psathyrella*, is the relatively pliant stipe. By virtue of the appendiculate cap margin the species logically belongs in the subgenus *Hypholoma*. Specimens which have lost all traces of a veil would be sought for in *Eupsathyrella*, but there are no species known to me in this subgenus which have this type of pleurocystidium.

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DESCRIPTION OF FIGURES

The drawings were made with the aid of a camera-lucida. The cystidia are reproduced at approximately 750 × and the spores 1650 ×.

FIG. 3. *a*, Spores of *Cortinarius subtortus; b*, spores of *Mycena rainierensis; c*, pleurocystidia and cheilocystidia of *Cortinarius subtortus; d*, cheilocystidia of *Mycena rainierensis; e*, spores of *Mycena fallax; f*, pleurocystidia of *Mycena fallax; g*, pleurocystidia of *Psathyrella alboalutacea; h*, spores of *Psathyrella alboalutacea.*

FIG. 5. *a*, Six spores of *Inocybe armoricana; b*, cheilocystidia of *I. armoricana; c*, four spores of *I. fastigiata* f. *alpestris; d*, cheilocystidia of *I. fastigiata* f. *alpestris; e*, eight spores of *I. griseo-lilacina; f*, three pleurocystidia of *I. griseo-lilacina; g*, four pleurocystidia of *I. laetior; h*, spores of *I. laetior; i*, spores of *I. leiocephala; j*, pleurocystidia of *I. leiocephala.*

FIG. 10. *a*, Pleurocystidia of *Inocybe oblectabilis* f. *decemgibbosa; b*, five spores of *I. oblectabilis* f. *decemgibbosa; c*, spores of *I. picrosma; d*, five pleurocystidia of *I. picrosma; e*, spores of *I. pyrotricha; f*, four pleurocystidia of *I. pyrotricha; g*, eight spores of *I. rainierensis; h*, pleurocystidia of *I. rainierensis; i*, spores of *I. suaveolens; j*, pleurocystidia of *I. suaveolens.*

FIG. 16. *a*, Pleurocystidium of *Psathyrella alboalutacea; b*, cheilocystidia of *Psathyrella alboalutacea; c*, spores of *Omphalina isabellina; d*, pilocystidia of *Omphalina isabellina; e* and *f*, caulocystidia of *Omphalina isabellina; g*, cheilocystidia of *Psathyrella candidissima; h*, spores of *Psathyrella candidissima.*

FIG. 18. *a*, Pleurocystidia of *Psathyrella candidissima; b*, spores of *Psathyrella caput-Medusae; c*, pleurocystidia of *Psathyrella caput-Medusae; d*, cheilocystidia of *Psathyrella caput-Medusae; e*, pleurocystidia of *Psathyrella Naucoria; f, g, i*, cheilocystidia of *Psathyrella Naucoria; h*, spores of *Psathyrella Naucoria.*

FIG. 20. *a*, Pleurocystidia of *Psathyrella rubicola; b, c*, and *e*, cheilocystidia of *Psathyrella rubicola; d*, spores of *Psathyrella rubicola; f*, pleurocystidia of *Psathyrella subalpina; g* and *h*, cheilocystidia of *Psathyrella subalpina; i*, cheilocystidia of *Psathyrella subtenacipes; j*, spores of *Psathyrella subalpina.*

FIG. 21. *a*, Pleurocystidia of *Psathyrella subtenacipes; b*, spores of *Psathyrella subtenacipes; c*, spores of *Clitocybe subvelosa; d*, spores of *Clitocybe gomphidioides.*

FURTHER INVESTIGATIONS ON THE PRESERVATION OF MOLD CULTURES[1]

Dorothy I. Fennell, Kenneth B. Raper, and May H. Flickinger

(WITH 1 FIGURE)

INTRODUCTION

Unprecedented study has centered upon the saprophytic molds during the past several years. This has resulted in large measure from the search for new antibiotic substances and from an intensive study of so-called deterioration processes In addition, there has been a generally quickened interest in these fungi in all fields where they occur as contaminants or where they seem to offer promise of producing desirable metabolic products. As the result of this increased study, workers in many laboratories have established culture collections of varying size and diversity. In maintaining these cultures, two considerations are uppermost, namely, (1) how to prolong viability, and (2) how to preserve morphological and physiological characteristics in unaltered form.

Much attention has been given to these matters at the Northern Regional Research Laboratory, and a considerable amount of information has accumulated since our collection of cultures was established in 1940. The purpose of this paper is to review briefly some of our experiences with different methods of culture preservation, and to consider particularly some recent results obtained with cultures preserved in lyophilized form.

Four years ago Raper and Alexander (**1945**) reported the successful application of the so-called lyophil process to the preservation of a wide variety of molds. Prior to this time, bacteriologists such as Shackell (**1909**), Hammer (**1911**), Rogers (**1914**), Swift (**1921** and **1937**), Elser, Thomas and Steffan (**1935**), and Flosdorf and Mudd (**1935** and **1938**) had developed this technique for preserving bacteria and immune sera, and had reported the successful preservation of cultures of streptococci and other pathogenic bacteria for periods up to 16 to 18 years. Wickerham and Andreasen (**1942**) had successfully used the method for preserving yeasts. Insofar as we are aware, our laboratory was the first to utilize the method successfully for the maintenance of mold cultures. Essentially, the process consists of (1) suspending conidia or other propagative cells in sterile blood serum or some other protein-rich medium, (2) dispensing the suspension into suitable tubes or vials, (3) freezing the suspension instantaneously at temperatures well below zero (– 40° to – 50° C.), (4) vacuum-desiccating the suspension from the frozen state, and (5) sealing under vacuum the tubes containing the desiccated preparations.

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Favorable viability tests at 20 to 24 months were reported by Raper and Alexander (**1945**) for many representative species of *Aspergillus* and *Penicillium*, for 19 species of the Mucorales belonging to 14 different genera, and for a wide variety of other molds representative of the Fungi Imperfecti and the lower Ascomycetes. Consistently negative results were obtained only from two members of the Entomophthorales, namely, *Entomophthora apiculata* and a species of *Conidiobolus*. In the same paper favorable viabilities up to 38 to 41 months were reported for a more limited series of cultures including a number of industrially important strains, and others which possessed unique cultural characteristics or were reported to be unusually short-lived. Good to excellent viability was obtained in most species, and poor viabilities, wherever they occurred, could generally be attributed to a thin initial spore suspension, or were observed to be associated with forms producing large or highly organized reproductive cells. In all cases, the cultures developing from the lyophil preparations were entirely typical of the strains under observation.

FIVE- AND SEVEN-YEAR LYOPHIL TESTS

Since that time additional viability tests have been made on these same cultures after approximately 5 and 7 years. Partial results of the latter tests are shown in table I. Results of early and inter-

TABLE I

VIABILITY OF LYOPHILIZED PREPARATIONS OF SELECTED MOLD CULTURES TESTED AT INTERVALS UP TO 85 MONTHS

Name	NRRL No.	Test No. 1		Test No. 2		Test No. 4		Test No. 6	
		Age in mo.	Viability	Age in mo.	Viability	Age in mo.	Viability	Age in mo.	Viability
Aspergillus flavus Link	693	2	+++	24½	+++	38½	+++	83½	++++
A. itaconicus Kinoshita	161	3½	++	26	++	41	+	84½	++
A. niger van Tieghem	3	4	+++	26	+++	41	+++	85	++++
A. niger van Tieghem	67	3	+++	25½	+++	40½	++	84½	++
A. niger van Tieghem	328	3	++++	25	++++	39½	++++	84	++++
A. niger (tan mutant)	P-88B	4½	+++	18	++++			70½	++++
A. oryzae (Ahlburg) Cohn	692			24½	++++	38½	++++	83½	++++
A. Sydowi (B. and S.) Th. and Ch.	P-35	2½	++++	24½	++++	38½	++++	70½	++++
A. terreus Thom	265	2	++++	24½	++++	38½	++++	83½	++++
Penicillium chrysogenum Thom	811	3½	++++	26	++++	40½	++++	85	++++
P. claviforme Bainier	1002	2½	++++	24½	++++	40	+++	68½	++++*
P. islandicum Sopp	1038	2	++++	24½	++++	38½	++++	72½	++++
P. lemoni Sopp	1042	2	++++	24½	++++	38½	++++	72½	++++
P. notatum Westling	824			18	++++	34	++++	76½	++++
P. purpurogenum var. *rubri-sclerotium* Thom	1064	4	+++	26	+++	40	+++	84½	++++
P. vinaceum Gilman and Abbott	739	2½	++	24½	+	38½	+	60	+
Gliocladium vermoeseni (Biou.) Thom	1752	2	+++	24½	+++	38½	+++	74	++
Blakeslea trispora Thaxter	1718	2½	+++	24½	+++	39	+++	83½	++
Mucor Ramannianus Moller	1559	2½	++++	24½	++++	39	++++	72	++++
Phycomyces Blakesleeanus (+) Burgeff	1554	2½	+++	24½	+++	39	+++	84	++
Phyco. Blakesleeanus (−) Burgeff	1555	2½	+++	24½	+++	39	+++	83½	++
Rhizopus delemar (Boid.) Weh. and Hanz	1472	2½	+++	24½	+++	38½	+++	75	+++
Rhizopus oryzae Went. and Pr. Geerl	395	4	+++	26	++	40½	+++	85	+++

++++ = Excellent viability
+++ = Good viability
++ = Fair viability
+ = Poor viability

* NRRL 1001 substituted for 1002.

mediate viability tests are reintroduced to provide a comparison of aged, intermediate, and newly processed cultures. Results of the five-year tests have been omitted since these were comparable in all cases with the seven-year viabilities; in fact, no marked decrease in viability was observed in any species during its entire period of lyophil preservation. Naturally, a certain degree of variability occurs. For example, two strains of *Blakeslea trispora* and two of *Phycomyces Blakesleeanus* showed only fair viability in the seven-year test after having been rated as good in all previous tests. On the other hand, cultures of *Aspergillus flavus* (NRRL 693) and *A. niger* (NRRL 3) were rated as excellent after having been scored as good in previous tests. A fluctuation in apparent viability of plus or minus 1 in our scoring system, which is admittedly arbitrary, should not be regarded too seriously. It may indicate either

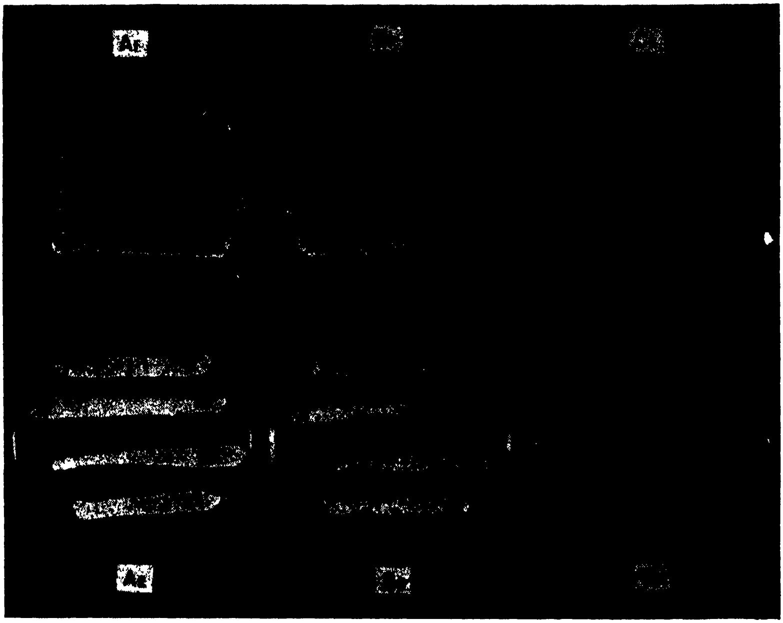

FIG. 1. Comparative growth of three representative molds, (A) *Penicillium notatum* NRRL 824 (Fleming strain), (B) *Aspergillus niger* NRRL 328, and (C) *Mucor Ramannianus* NRRL 1559, inoculated with spores from (1) current agar stocks and (2) five-year lyophilized preparations. Substrate: Czapek's solution agar. Incubaton: two days at 25° C.

a difference in evaluation of growth by different observers, or it may reflect differences in individual lyophil preparations, e.g., variation in the spore density of different fractions of the original suspension or differences in the extent of vacuum desiccation.

It is evident from the data of table I that the cultures under observation have in most cases retained their viability almost unchanged during a storage period of seven years. The viability of cultures of *Blakeslea* and *Phycomyces* may be on the decline; but simply to have maintained viable cultures of the former without transfer for seven years represents a considerable achievement. The recorded poor viability of *Penicillium vinaceum* and *Aspergillus itaconicus* is unquestionably due to the low spore content of the suspensions processed. For an *Aspergillus*, the latter species is unusually short-lived when maintained in agar slants in the conventional manner.

Whereas relatively few cultures have been preserved in a desiccated state for seven years, the entire mold collection as it then existed (numbering about 1850 different strains) was so preserved in 1942, and these preparations are now six or more years old. Tubes have been opened, as occasions demanded, and with very few exceptions viabilities have been regarded as entirely satisfactory (FIG. 1). Raper and Alexander in 1945 presented results of tests made at 1½ to 2 years on 140 representative cultures from the 1942 preservations, including 41 Aspergilli, 40 Penicillia, 29 members of the Mucorales, and 31 miscellaneous forms. In 1947 a second set of tubes from the same series of 140 cultures was opened. Results obtained in this five-year test, in the main, duplicated those previously observed. In 14 strains a marked decrease in or lack of viability was recorded, while in 4 strains the rating indicated greater viability than had been previously reported. When duplicate preparations of nine of the strains showing reduced or no viability were examined, results equal to or approaching those at the two-year interval were observed in four cases, whereas in three the decreased viabilities indicated in the primary five-year tests were confirmed. In two others, cultures were found to be nonviable: in one case (*Penicillium puberulum*) the lyophil preparation was found to be suboptimal in character with very poor vacuum and containing a soft spongy pellet—while in the other (*Chaetocladium Brefeldii*) no physical factors which might have adversely affected viability were observed. Tests were not repeated on five of the fourteen above-mentioned strains since no preparations of the original processing date were available for testing.

COMPARISON OF DIFFERENT METHODS OF CULTURE PRESERVATION

In order to evaluate various methods of culture preservation, a rather extensive program was initiated early in 1946. The Mucorales were selected as test organisms. Methods investigated included: (1) preservation in plain agar slants, (2) preservation in fertile garden soil, (3) preservation under a mineral oil seal, and (4) preservation in lyophilized form.

The first method represented the conventional procedure practiced in most laboratories, with cultures being grown on a suitable agar medium—in this case malt extract agar. The second method represented an adaptation of a technique long employed for the preservation of bacteria, particularly the anaerobic spore-forming clostridia. As employed by us, the method followed the procedure recommended for the maintenance of molds by Greene and Fred in **1934**. With limited modifications, preservation in soil is widely employed in industrial laboratories for the maintenance of penicillin-producing molds and other economically important types. The technique of keeping cultures under mineral oil was developed for the preservation of bacteria by Ungermann (**1918**), Michael (**1921**), Morton and Pulaski (**1938**), and others, and was, apparently, first used successfully for the maintenance of fungus cultures by Sherf in **1943**. More recently it has been employed by Wernham (**1946**) and Wernham and Miller (**1948**). Conservation of fungus cultures under oil has been warmly recommended by Buell and Weston (**1947**).

In preparation for the comparative tests, four-year-old lyophil tubes of 245 cultures belonging to the Mucorales were opened and recultivated on malt extract agar. The different types of cultures used for the subsequent comparison of methods were inoculated or prepared from spores obtained in this recultivation. For each of the 245 strains, the following number and types of cultures were prepared simultaneously: two plain malt extract agar slants, a similar slant flooded with a layer of sterile mineral oil after satisfactory growth and sporulation had occurred, a soil culture, and six lyophil preparations. All cultures were subsequently stored at 4° C.

When the cultures were somewhat more than two years old, streak plates were made on malt extract agar from all preparations, care being taken insofar as possible to use approximately equal amounts of inocula from the different types of cultures. No growth resulted from 72 of the dried-out agar slant cultures. Forty-seven of the oil-covered slants and sixteen of the soil cultures were likewise negative. In every case, however, lyophilized cultures yielded growth characteristic of the strains under observation, and in 235 strains (96 per cent), the viability was rated as either excellent or good. In four cases viability was quite poor. Good or excellent viability was also recorded for 135 of the soil preparations, 123 of the oil-flooded slants, and 60 of the plain agar slants. These data are summarized in table II.

TABLE II

VIABILITY OF MEMBERS OF THE MUCORALES CONSERVED FOR 2 TO 2½ YEARS BY DIFFERENT METHODS OF CULTURE PRESERVATION

Viability / Method	Excellent	Good	Fair	Poor	Negative
Lyophilized preparations	205	30	6	4	0
Soil cultures	32	102	65	30	16
Oil-sealed agar slants	26	95	49	28	47
Malt agar slants	13	49	66	45	72

Whereas no strict correlation was observable, a general relationship was found to exist between viability and species identity. Species of *Blakeslea, Chaetocladium, Circinella,* and *Cunninghamella* showed the greatest percentage of non-viability, particularly in the plain agar and in the oil-covered slant cultures. For example, of 17 strains of *Circinella* tested, only 5 remained viable under oil and an equal number, but not identical strains, on plain agar slants. Of six strains of *Cunninghamella echinata* tested, all failed to grow from the oil-covered slants, and only one of the plain agar slants yielded a viable culture. Two strains of *Chaetocladium Brefeldii* likewise failed to grow from oil-covered cultures but grew poorly from plain agar slants, whereas the single strain of *Blakeslea trispora* included in the test proved to be non-viable in both types of cultures. In all of the above cases, good to excellent viability was obtained from lyophilized cultures of similar age.

Based upon these tests, which are summarized in table II, it would appear that the Mucorales can be maintained most satisfactorily in lyophilized form, with soil cultures, oil-covered agar slants, and plain agar slants representing progressively less favorable methods.

APPLICABILITY OF THE LYOPHIL PROCESS TO THE PRESERVATION OF OTHER FUNGI

Since the fungus cultures contained in the NRRL Collection represent, for the most part, saprophytic molds important to agriculture and the fermentation industries, our data on the applicability of the lyophil process to the preservation of fungi were largely limited to molds of this type.

Information regarding the preservation of other groups of fungi was deemed highly desirable. Additional species chosen for exploratory investigations included a limited number of aquatic Phycomycetes, representative wood-destroying Hymenomycetes, and several fungi pathogenic to man and animals. The Phycomycetes were provided by Charles Drechsler, Bureau of Plant Industry, Beltsville, Maryland; the Hymenomycetes by Ross W. Davidson, also Bureau of Plant Industry; and the pathogenic forms by C. W. Emmons, National Institute of Health, Bethesda, Maryland.

The strains belonging to each general group were cultivated on suitable media and lyophilized by our usual methods (see page 2; also Raper and Alexander, **1945**). Desiccated preparations were tested for viability immediately after processing, with the following results:

Aquatic Phycomycetes: Nine strains of *Pythium,* representing as many species, and one culture of *Plectospira myrianda* were tested with negative results. Earlier, in cooperation with Dr. John R. Raper, University of Chicago, attempts had been made to lyophilize four strains of *Achlya,* including two strains each of *A. bisexualis* and *A. ambisexualis,* in suspensions made both with blood serum and with a thin starch paste. Negative results were obtained in these trials also and are confirmatory of those reported in Buell and Weston (**1947**) for members of this group.

Hymenomycetes: The cultures of wood-destroying fungi included three species of *Polyporus*, two each of *Poria* and *Fomes*, and one each of *Stereum* and *Lenzites*. In our cultures, all but two of these produced conidia, oidia, or chlamydospores—all representing types of spores thought to provide suitable material for lyophil desiccation. The two exceptions were *Stereum rameale* and *Fomes pinicola*. Lyophil preparations of all strains showed excellent viability except the two just mentioned and one additional strain, *Fomes annosus*. Of these, the two strains of *Fomes* showed limited viability. In three successive trials the strain of *Stereum rameale* failed to survive the lyophil process. This was considered to result probably from an observed absence of suitable propagative cells.

Pathogenic Fungi: Among the pathogenic fungi received from Dr. Emmons were seven strains of *Trichophyton*, representing four species, and one strain each of *Candida albicans*, *Sporotrichum schenckii*, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and *Histoplasma capsulatum*. Excellent growth was obtained from lyophil preparations of all but the last three strains. Of these, *Cryptococcus neoformans* showed good viability and the remaining two only fair. The apparent reduced viability of the latter may have resulted in large measure from a dearth of spores in the processed suspensions, or from the possible use of a suboptimal medium during recultivation.

The reader may justifiably question the significance of viability tests made immediately following lyophil preservation. However, we have consistently observed during an eight-year period that, once cultures have been lyophilized successfully, they retain their viability with little or no apparent decline. When difficulties are experienced, they are encountered during the freezing and drying process.

DISCUSSION

The preservation of cultures in soil, as oil-covered agar slants, or in lyophilized form necessitates the expenditure of greater initial time and effort than maintenance of the same cultures on plain agar slants. Justification for such methods must rest, therefore, either upon prolonged viability or upon increased morphological or physiological stability. While our experience does not warrant categorical statements, it is becoming increasingly evident that among the molds producing abundant aerial spores, or conidia, viabilities are as a rule greatly prolonged by lyophil conservation. Furthermore, the new growth resulting from such cultures must develop from the actual spores or cells originally processed; hence the danger of variation is negligible except for the possibility that such may arise as a result of the freezing and drying process. It is significant that in eight years we have not observed a single instance where colonies developing from lyophilized spores differed in any detectable degree from those resulting from normal undesiccated spores of the same strain. Similarly, we have encountered no case where biosynthetic capacity has diminished as a result of lyophilization. On the contrary, in the production of itaconic acid by *Aspergillus terreus*, cultures preserved in desiccated form have maintained their capacity to produce this acid, whereas cultures maintained on agar slants by periodic transfer have shown a marked decrease in biosynthetic capacity (L. B. Lockwood, personal communication). The same may be said of the penicillin-producing species, *Penicillium notatum* and *P. chrysogenum*. However, too sweeping claims should not be made, and attention is called to the recent paper by Atkin *et al.* (**1949**), wherein lyophilization is reported to reduce the capacity of brewers' yeast to synthesize essential vitamins.

Most of the mold cultures which we maintain are well adapted for lyophil preservation since they produce abundant aerial spores or conidia. A limited number, however, cannot be satisfactorily maintained in this manner. These include forms which produce few or no conidia or other firm-walled propagative cells, and certain species which produce spores that are unusually large or highly organized. Recent experiences with certain aquatic Phycomycetes emphasize the unsuitability for lyophilization of zoospores and other reproductive structures in these forms. Repeated tests have demonstrated that vegetative mycelia, fragmented or otherwise, cannot be successfully conserved, and our experience with a limited number of wood-destroying species has confirmed this observation.

Species producing oidia or other propagative cells were successfully processed; whereas in the absence of such cells, conservation by the freeze-drying technique was not realized. The importance of cell-type to successful application of the lyophil process is beautifully illustrated by slime molds belonging to the genera *Dictyostelium* and *Polysphondylium*. Attempts to conserve the amoeboid vegetative cells have been wholly unsuccessful, whereas the capsule-like mature spores of all species tested are admirably suited for this type of preservation. Preparations up to eight years old (the maximum of our tests), if added to or preserved with a suitable bacterial host (Raper, 1937), give rise to luxuriant and wholly typical cultures when recultivated upon suitable substrata. Such cultures develop almost as rapidly as others seeded with fresh spores.

Buell and Weston (1947) have correctly drawn attention to the limitations of the lyophil process as a means of conserving collections of diverse fungi and have emphasized the utility of other methods, notably conservation under oil. While we feel that they tend to over-emphasize the difficulties to be encountered in applying the lyophil process, we are in essential agreement with them and fully recognize the vital role of other methods of conservation. As a matter of fact, and depending upon the nature and importance of the strains to be preserved, any one or all of the four methods of conservation discussed above may be used advantageously. Each has its strength and its weakness, which we believe can be summarized as follows:

The agar slant method: Transfers, in whatever numbers are required, can be made quickly, and one can observe the fungus throughout its growth and development. Such cultures are not long-lived, however, and there is a constant danger of cultural or physiological variation with each period of regrowth, particularly if a degree of selection enters into the choice of the inocula used to establish succeeding transfers.

The oil-seal method: The viability of cultures is generally increased, and the method is especially applicable to the conservation of mycelial or non-sporulating forms. Mites are effectively controlled. It is receiving enthusiastic acceptance by an increasing number of investigators. However, preparation requires additional labor and the resulting sealed cultures must be stored in an upright position at all times.

Soil preservation: Culture viability is usually substantially increased, and, if proper care is observed in handling, satisfactory and uniform subcultures can be obtained from the same preparation over long periods of time. As with the oil-seal method, preparation requires additional work, and in this case it is impossible to observe growth or to detect contamination without recultivation.

Lyophil preservation: Culture viability is greatly prolonged; new cultures arise from the spores originally processed, and the preparation of multiple tubes provides a source of uniform inoculum over an indefinite period; the possibility of contamination by mites or microörganisms is eliminated by sealing preparations in glass; strain variation is minimized through infrequent recultivation; the diminutive proportions of the finished preparations are conserving of valuable storage space. The method's most serious drawback is its inapplicability to the conservation of many types of fungus cultures which do not produce small, firm-walled spores or conidia. A less serious objection is the necessity of opening a new preparation each time a new culture of "lyophil" origin is required. Finally, preservation in lyophil form entails a greater amount of effort than any of the preceding methods.

SUMMARY

If a culture lends itself to lyophil preservation, we believe this to constitute the most reliable means of maintaining it in unaltered form over long periods of time. In the long run it is also the least time-consuming. If the culture cannot be so preserved, one must rely upon alternative methods. Preservation in soil offers certain favorable advantages, but this method—like lyophil preservation—is not applicable to the maintenance of mycelial forms. If the culture is strictly mycelial, or if very few spores are produced, preservation under oil generally affords the best means of conservation.

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MORPHOLOGY OF DISCISEDA CERVINA

SULTAN AHMAD

(WITH 3 FIGURES)

The genus *Disciseda* was proposed by Czerniaiev (**1845**) for a plant in which the exoperidium had persisted as a disc at the base. As he neither described the relevant microscopical characters nor gave any diagrams, the genus remained obscure and unrecognized for a very long time. Morgan (**1892**) described the genus *Catastoma*, characterized by the basal mouth, the capillitium, which consisted of short separate threads, and the apical collar-like remains of the exoperidium. Hollós (**1903**) dug out the generic name *Disciseda* and claimed that it was co-generic with *Catastoma* of Morgan. His labors, however, were not rewarded for a long time, as several leading mycologists preferred to use the very appropriate name *Catastoma* (basal mouth). Now, however, mycologists have begun to recognize *Disciseda* as the valid generic name on grounds of priority, even though Lloyd (**1904**) protested strongly against this tendency.

The genus is considered by several workers as curious and unique among the Gasteromycetes in having a basal mouth. Lloyd (**1918**), however, writes of *Catastoma ater* that "it is not a true *Catastoma*, . . . in the 'type idea' with 'mouth down,' but there are several species of *Catastoma* that will not stand that test." Recently Cunningham (**1946**) also states that that is not a universal feature and that in *Disciseda cervina, D. australis, D. anomala, D. verrucosa,* and probably others, the stoma develops apically in the usual manner. Lohwag (**1930**) finds it difficult to explain the basal mouth morphologically. The explanation offered by him is that the endoperidium at the base is torn from the "Myzelium" whereby a hole or at least a weak spot originates which later wears into an aperture.

As most of the species grow wholly or partially buried under the surface of the ground, nothing is known about the early developmental history of the fungus. The description of the various species is based only on the gross morphological features of the mature fructification. The different statements concerning the development of the mouth at the apex in several of the species are merely based on guess-work. The only reference to even the structure of the exoperidium and gleba of any species is a brief statement by Coker and Couch (**1928**) in connection with the description of *D. candida*.

The only representative of the genus in the West Panjab is *D. cervina* (Berk.) Hollós, which is very common in the open sandy wastes. As this species was among those in which Cunningham has reported an apical mouth, a close observation of the fungus was undertaken and an effort was made to procure young sporophores at all stages of development. Fortunately several young plants were collected in the summer of 1948, growing in a locality from which hundreds of mature specimens had been obtained in the past.

Several of these plants were fixed immediately in formalin-acetic-alcohol, the only fixative available at the time. The sections were cut 5-10 μ thick and stained with iron hematoxylin and phloxine.

OBSERVATIONS

The young sporophores are always obovate or turbinate with the apical part more or less exposed. They are attached by a well developed mycelial cord at the base The only species in the vicinity with the sporophores of which these could be confused are *Lycoperdon pusillum* and *Lanopila wahlbergii.* The former is always epigeal and the latter, though hypogeal, has a slightly different color and avoids loose sandy soil. With a little experience in the field one can easily recognize the young sporophores of *D. cervina* by their form, color and peculiar behavior during later stages of growth.

The first thing in the young sporophores which attracts attention is the floccose mycelium arising from the exposed top portion. These sporophores when allowed to develop *in situ* disappeared from sight during the night. It was very difficult to guess what had happened to them. It was first thought that they had probably been eaten up by some rodent, but there were no fragments lying nearby to confirm this conjecture. After experiencing the same difficulty for two or three days successively a few plants were covered with small earthen cups but the result was still the same. Very much puzzled at this, the soil under the cups was carefully dug out and then it was discovered that the plants had buried themselves under the soil in all cases. A more careful observation revealed that the hyphae of the floccose mycelium at the top ramify and enclose numerous grains of sand and disappear from sight. Among two or three dozen plants unearthed at different intervals of time, it was found that the formation of the hyphal covering enclosing sand particles progresses from the apex downwards till the whole plant is covered with a sandy case. The sandy case is more or less uniform in thickness and so the plants retain their original form.

The sandy case was never seen to split in a circumscissile manner described and figured by Morgan. On the other hand, it always crumbles from the base upwards after the plants have been unearthed by wind. When most of the loose basal part is lost the heavy top assumes a basal position. In the plants unearthed after about ten days the basal mycelial cord was still intact. In some, however, it appeared to have gelatinized at the base, as it was easily pulled out, leaving a pore—the mouth or stoma—at the base.

Mycelial cord. The mycelial cord consists of a central medulla, a middle sub-cortical region, and an outer cortex. The medulla consists of thick, wide-lumened hyphae intermixed with numerous thin-walled hyphae. The sub-cortical zone is formed of very compact and closely interwoven hyphae. The outermost hyphae of the cortex branch and enclose numerous particles of sand.

Development. The young sporophores develop at the apex of a mycelial cord. Sometimes two growing close together become fused with one another. At a very early stage of development each consists of loosely interwoven hyphae continuous with those of the parent strand. The thick, wide-lumened hyphae of the medulla and the hyphae of the cortex do not take part in the formation of the young sporophore. In the center of the homogeneous tissue are later differentiated small cavities which gradually progress towards the periphery. The development corresponds in all essential respects to the *lacunar type* as described by various investigators for other members of the family Lycoperdaceae.

Exoperidium. The hyphae forming the primordial tissue radiate towards the periphery; they are parallel and wide-lumened. These hyphae become closely septate and the cells become swollen and compacted to form pseudoparenchymatous tissue. In a fully formed plant this layer is 400–500 μ thick and consists of several

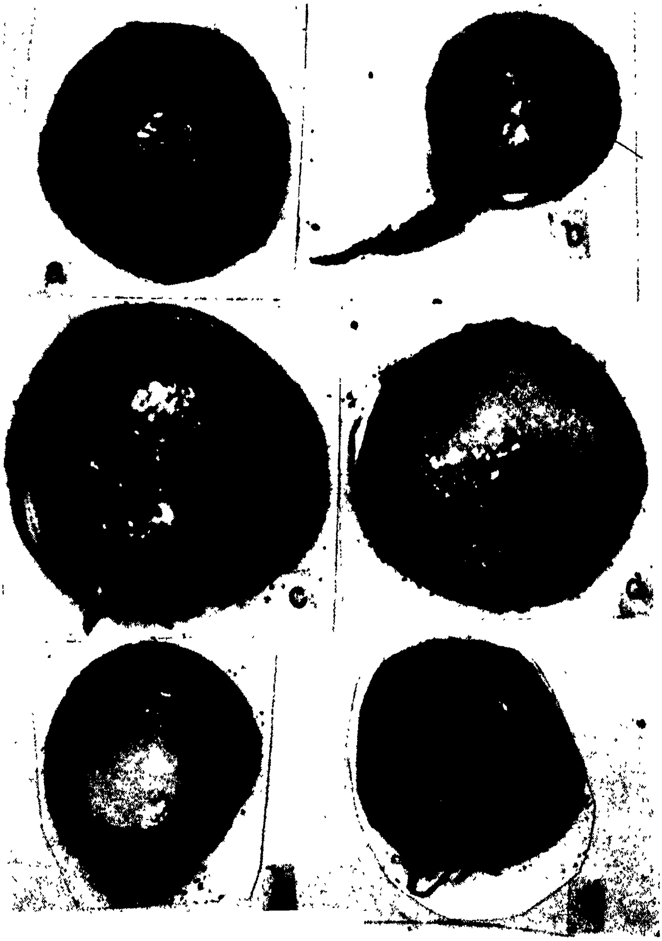

FIG. 1. *Disciseda cervina.*

layers of thin-walled cells. This layer though continuous around the upper part of the fruitbody is poorly developed at the top. At the base, however, it is interrupted by the mycelial cord.

After the pseudoparenchymatous layer has been organized, a second or mycelial layer begins to form. This is, however, completed after the differentiation of the endoperidium has taken place. The mycelial layer is composed of long, intertwined, partially gelatinized hyphae which arise from the exposed top portion of the sporophore. The hyphae ramify in the surrounding soil and enclose numerous sand particles to form a small cap at the apex (FIG. 1, *a–e*). In the course of time the mycelial layer progresses downwards, ultimately forming a complete sandy case around the sporophore (FIG. 2, *a*). It is only incomplete at the base from where the mycelial cord comes out (FIG. 1, *f*).

The pseudoparenchymatous layer is very poorly developed at the top and several hyphae of the endoperidium are also seen passing through it into the mycelial layer. On the sides below the apical disc there is absolutely no connection between the mycelial layer and the hyphae of the endoperidium. This intermixing of the hyphae of the mycelial layer and the endoperidium at the top accounts for the persistence of the apical collar or disc.

The cells of the pesudoparenchymatous layer collapse at maturity and form a thin membrane on the inner surface of the sandy case. The disorganization of this layer at maturity loosens the sandy case all round excepting at the apex, as explained above. When the plants are exposed by wind they lie scattered on the surface of the ground with the sandy case still intact. The loose basal part gradually crumbles by the attrition of sand particles until only the apical disc is left (FIG 2, *b–i*).

The top-heavy plant now easily gets overturned and the collar or the disc appears to be basal. The appearance of the "*exoperidium*" as collar or disc depends on the size of the case crumbled at the time of collection. In very old, weathered plants there is only a small disc left at the base, indicating the region where the union was strongest (FIG. 2, *j–w*).

Gleba. The hymenial cavities originate and increase in number exactly as in other members of the family. The ground tissue grows by the expansion of its elements and the formation of new

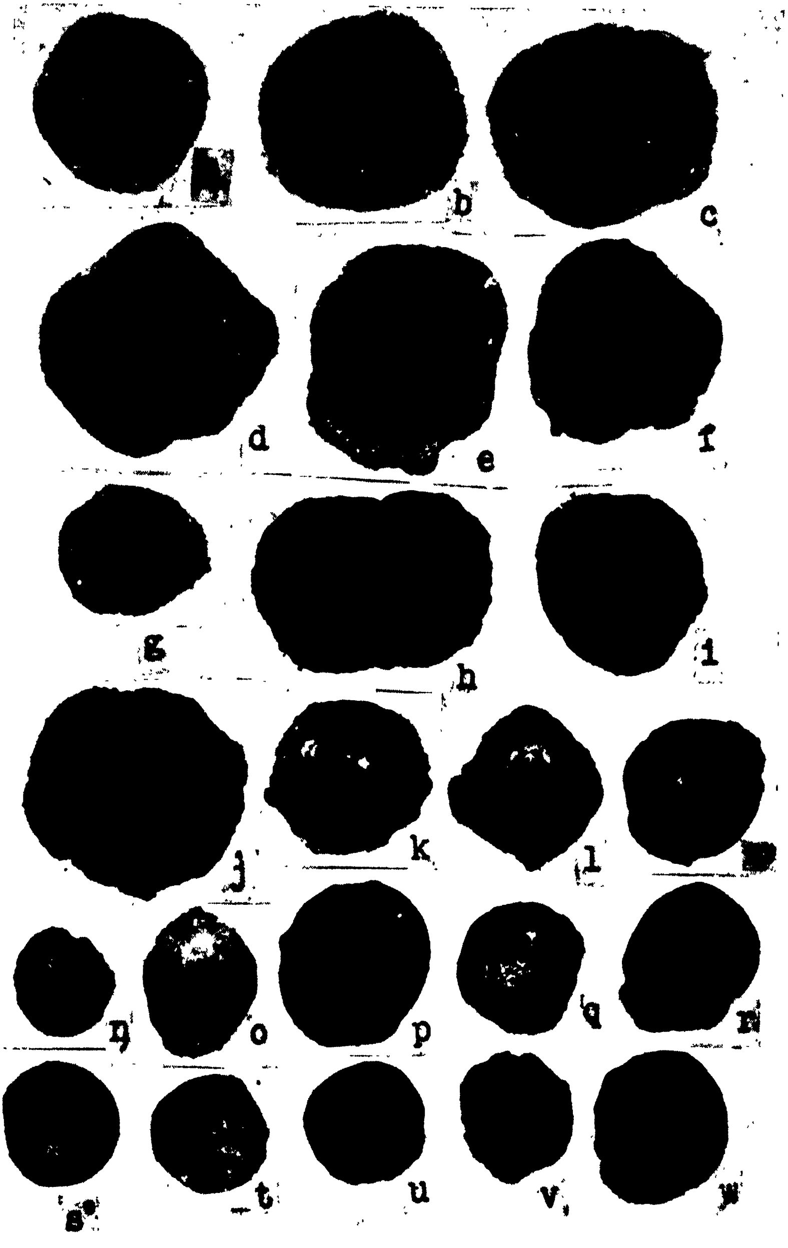

FIG. 2. *Disciseda cervina.*

tramal plates. Most often stout hyphae pass from one side of the hymenium to the other across the hymenial cavity. As pointed out by Swartz (**1933**) these hyphae clearly suggest that the formation of cavities results entirely from disintegration of the primordial hyphae.

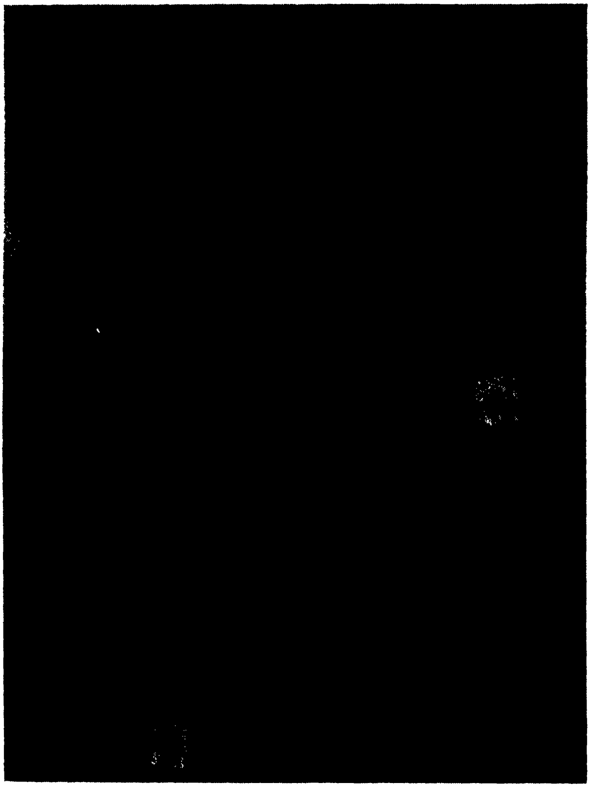

FIG. 3. *Disciseda cervina.*

The basidia, arranged in compact hymenia, are short and pyriform, bearing four spores apically on long sterigmata. The thickening on the spores develops after they have been detached from the sterigmata. After the spores are formed, a rapid disintegration of all the remaining glebal hyphae and basidia takes place, leaving nothing excepting spores and capillitium. The capillitial threads arise from the tramal hyphae and not in the manner described by Cunningham (**1926**) for *Lycoperdon depressum.* At maturity the capillitium breaks up into short segments.

Endoperidium. When the development of the mycelial layer has not yet progressed even half way down, the endoperidium becomes differentiated (FIG. 3, *a*). It is formed immediately below the pseudoparenchymatous layer of the exoperidium. In earlier stages this region appears as an undifferentiated outer part of the gleba bordering the hymenial cavities. Later there are differentiated in this region numerous stout interwoven hyphae which become arranged tangentially and form the fundament of the endoperidium. At this time it is about 150 μ thick and the glebal tissue has not yet disintegrated.

The endoperidium is interrupted at the base by the mycelial cord, which occupies an area of about 1.16 mm. in size. When the basal part of the mycelial cord penetrating the exoperidium and the endoperidium gelatinizes, an opening is left at the base which serves as a mouth or stoma.

DISCUSSION

The genus *Disciseda* is included in the family Lycoperdaceae. According to Cunningham (**1946**, p. 28) it is "the most primitive genus, as is shown by its external sand-case exoperidium and somewhat hypogean habit." This statement is evidently not based on any ontogenetic investigation of the young stages. There is no doubt that in the lower members of the group we come across a peridium which is always a simple undifferentiated layer of the ground tissue, but in *Disciseda* the peridium consists of two distinct layers—an exoperidium and an endoperidium. The exoperidium is further differentiated into an inner pseudoparenchymatous and an outer mycelial layer.

According to Lohwag (**1928**) the number of peridial layers is not an absolute criterion of higher organization but depends on the form of the fruitbody. Still, if the peridium must be taken as of some systematic value, it will be seen that *Disciseda* is more highly organized than all the other members of the family Lycoperdaceae. The differentiation of the peridium into several layers takes place according to varied and important ecological functions.

It may also be true, as pointed out by Gäumann and Dodge (**1928**), that the primitive Gasteromycetes are mostly hypogeous but with a strong tendency to elevate the gleba above the earth to secure the dissemination of their spores by wind or insects. In assigning *Disciseda* a primitive position on this ground, Cunningham has overlooked the most fundamental fact that the subterranean habit has developed independently among several different groups of fungi which are the most highly organized.

In the Gasteromycetes a pulverulent gleba, well developed capillitium, and a mouth for the dissemination of spores by wind are characters closely associated with an epigeous mode of life. It is, therefore, safe to assume that *Disciseda* has evolved from some epigeous ancestors and that the subterranean habit is of eco'ogical rather than of phylogenetic interest. It appears to me that the hypogean habit, in plants growing in hot sandy areas, has developed in response to the extremely xerophytic conditions of life. As the loose sandy soil has little power to retain water and the superficial layers of soil soon become dry even after a heavy shower, the plants have secondarily retired under soil. Under these conditions, we see that the most successful forms are those which originate the deepest of all and these are *Montagnites arenarius*, *Tulostoma volvulatum*, *Schizostoma mundkuri*, *S. laceratum*, *Podaxis pistillaris* and *Phellorina inquinans*.

If we imagine a large number of species growing on the edge of a drifting sand dune and during the course of ages regularly buried under sand, it would become clearer how they acquire their present-day mode of life. At first the layers of sand were probably not thick enough or were washed away and the dispersal of spores was effected in a normal fashion. When the layers of sand became sufficiently thick only such plants escaped elimination as could produce some mechanism to elevate their sporocarp for the dissemination of spores by wind. Some plants, like species of *Geaster*, developed a highly specialized peridium, while others developed a very long woody stipe for this function. The former set of species was confined to the superficial layers of soil while the latter was adapted to very deep layers. The woody stipe is an absolute necessity for the breaking of the thick layers of soil above the sporophore. It is interesting to find that under similar environmental conditions even a *Coprinus*-like *Montagnites* developed a long woody stipe similar to other Gasteromycetes living in the same habitat.

The same thing has been observed by the late Dr. W. H. Long (**1942**) in his study of the different species of *Geaster.* He writes as follows: "All species of *Geaster* in my territory are hypogeous when young and remain so until expansion. I have never seen a truly epigeous *Geaster* in the dry southwestern regions. The reason for this is evident, in that our climate below 8000 ft. elevation is too dry for species of *Geaster* to grow on the top of the ground as some species do in the humid regions of the north and east. This need of moisture also explains their presence under trees and shrubs where the moisture is retained until these fungi mature."

The hypogeous species originating in the superficial layers of sand stand at a distinct disadvantage as compared to those mentioned above but *Disciseda cervina* has specialized in a particular direction. The plant has developed an efficient mechanism for "burrowing" into the loose sand and at the same time forming a case of sand which serves it as a moist chamber. This minimizes the chances of sudden drought overtaking the developing sporophores. The mechanism is so efficient that of the two or three hundred individuals gathered at different times there was not even one in which the gleba had not reached proper maturity.

Another specialization consists in making use of the sandy case in the dissemination of the spores. The basal mouth which developed as a necessity is definitely a disadvantage but the heavier apical disc shifts it to the top so that it can play its normal role in the life of the plant. This is the most curious phenomenon observed in this species, and is unparalleled in the whole group of fungi.

In *Lycoperdon pusillum,* an epigeous species growing side by side with the *Disciseda,* one finds about fifty per cent of the plants with growth arrested at some stage before the proper ripening of the gleba. The same thing has been observed in *Lanopila wahlbergii*, which though hypogeal occurs in the superficial layers of the soil and without any specialization. It is doubtful if the latter species can be classed as among the true arenicolous species mentioned above, as it avoids the loose sandy soil and seeks the shelter of the shrubs.

It has been customary to include all the Gasteromycetes with capillitium and pulverulent gleba in the family Lycoperdaceae. Different functions have been assigned to the capillitial threads by different workers. According to de Bary (**1887**) and others they help in the dispersal of spores but according to Swartz (**1933**) they are associated more with the disposal of waste products than with the dispersal of spores. Whatever their function may be in the earlier stages, one thing is certain and it is that at maturity they prevent the collapsing of the peridium when it is pressed by some external agency. They ensure thus the dispersal of spores at intervals in puffs or clouds.

The form of the capillitium varies in the different genera of the family. In *Lycoperdon* and *Calvatia* the capillitium is well developed and consists of long, branched, septate, and intertwined threads. In *Bovista* and *Mycenastrum* the capillitium consists of short separate threads. In *Disciseda*, *Abstoma*, and *Lanopila* the capillitium is of the *Lycoperdon*-type but at maturity it breaks up into short segments. The significance of the different forms of capillitial threads in one family is obscure, especially when they are supposed to undertake the same function.

The genus most closely related to *Disciseda* is *Abstoma*. The genus *Abstoma* resembles *Disciseda* in every respect excepting the absence of a mouth. The reticulate spores do not serve as a distinguishing feature, as Zeller (**1948**) has recently described a species with verrucose spores. Until the development of the genus is studied it would be difficult to state how the mouth has become eliminated.

In the present state of our knowledge, and assuming that the hypogeal forms have gradually evolved from the epigeal forms under changed ecological conditions, the genus *Disciseda* would be regarded as a very highly specialized member of the family Lycoperdaceae.

SUMMARY

1. *Disciseda cervina* is semi-hypogeal in the earlier stages but becomes completely hypogeal by developing a mycelial layer round it.

2. The sporophore is attached by a well developed mycelial cord of complex structure.

3. The development is of the *lacunar type*.

4. The peridium consists of three layers: mycelial layer, pseudoparenchymatous layer and the endoperidium.

5. The mycelial layer forms around the entire fruit-body a sandy case which crumbles down when the plant is exposed by wind. The sandy case persists as an apical disc and helps in inverting the plant.

6. The mouth is formed at the base by the gelatinization of the basal part of the mycelial cord penetrating the exoperidium and the endoperidium. When the top-heavy plant becomes inverted the mouth assumes an apical position.

7. The structure of the gleba and the origin of the capillitium is of the same type as described for other members of the family.

8. The hypogeous habit has been secondarily acquired under changed environmental conditions.

9. The genus *Disciseda* is a highly specialized member of the family Lycoperdaceae.

The writer is deeply indebted to Dr. Nazir Ahmad and Sher Ahmad Khan Lodhi for aid in the preparation of the manuscript and to Dr. Donald P. Rogers for reading the typescript.

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EXPLANATION OF FIGURES

FIG. 1. Young sporophores showing different stages in the progression of the mycelial layer. In *f* only a small portion round the mycelial cord remains uncovered. × 2.

FIG. 2. *a.* Sporophore wholly covered with a sandy case. The basal mycelial cord was broken by careless handling. *b–i.* Mature plants showing gradual crumbling of the sandy case from the base upwards. The basal orifice is clearly seen in some. *j–w.* Several plants collected in February 1949 showing the marked reduction in size of the apical disc due to weathering. × 2.

FIG. 3. *a.* Semi-diagrammatic section of young fructification showing the formation of the mycelial layer at the top. The endoperidium is fully differentiated at this stage. The two peridium layers are interrupted at the base by the mycelial cord. *b.* Diagrammatic section of a mature fructification. M.L. Mycelial layer; Ps. Pseudoparenchymatous layer; End. Endoperidium; Gl. Gleba; M.C. Mycelial cord.

PRODUCTION OF HYDROCYANIC ACID BY CULTURES OF A BASIDIOMYCETE[1]

WILLIAM J. ROBBINS, ANITA ROLNICK, AND FREDERICK KAVANAGH

(WITH 3 FIGURES)

In the course of investigations on the production of antibacterial substances by Basidiomycetes, an unidentified fungus (B841) from white cedar[2] was grown on a liquid medium in 2800 ml. Fernbach flasks in a large cabinet maintained at 25° C. It was observed that *Penicillium funiculosum* grew unsatisfactorily in this cabinet on agar slopes in test tubes (FIG. 1). Investigation demonstrated that the poor growth of the *Penicillium* was caused by a volatile toxic substance produced by the B841 fungus. The toxic substance is believed to be HCN.

Although HCN was first reported in *Marasmius oreades* by v. Losecke in 1871 (**5**) and has since been demonstrated in the sporophores of a number of Basidiomycetes (**1**, **2**, **3**, **4**, **6**), the production by fungous mycelium in culture of sufficient amounts to interfere with the growth of neighboring cultures of other organisms has not been reported.

The cabinet—7 feet high, 6 feet wide and 2 feet deep—contained 7 shelves with 48 Fernbach flasks each inoculated with fungus B841 and distributed as follows: 16 on the first shelf, 6 on the second, 3 on the third, and 23 on the fourth. Each Fernbach contained 1 liter of a corn steep medium (**7**). The dry weight of the mycelium of fungus B841 in a Fernbach flask was approximately 3.5 g. and the surface area about 255 sq. cm. The cultures of the *Penicillium* were incubated on the fourth shelf. The doors of the cabinet were not hermetically sealed and were, as a rule, opened once or twice each day of the week except Saturday and Sunday. Under these circumstances, the accumulation of sufficient HCN or other volatile toxic material to become inhibitory was unanticipated.

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[1] This investigation was supported in part by the Howard Bayne Research Fund of The New York Botanical Garden.

[2] We are indebted to Dr. Dow V. Baxter for the culture of this fungus.

The inhibitory action of fungus B841 was confirmed by covering one of the cultures in a Fernbach flask with a bell jar. Agar slants inoculated with *Penicillium funiculosum* were incubated under the bell jar and duplicate cultures were incubated outside. The media used were 2 per cent malt agar, thiamine-peptone agar [3] and an agar with $NaNO_3$ furnishing the nitrogen.[4] At intervals the bell jar was lifted and aerated to avoid anaerobic conditions. The cultures of fungus B841 were approximately 3 months old but the culture liquid had been decanted and replaced with fresh sterile medium at intervals of about 4 weeks. The fungus mat was heavy, white and vigorous; no fruiting bodies were present. The growth

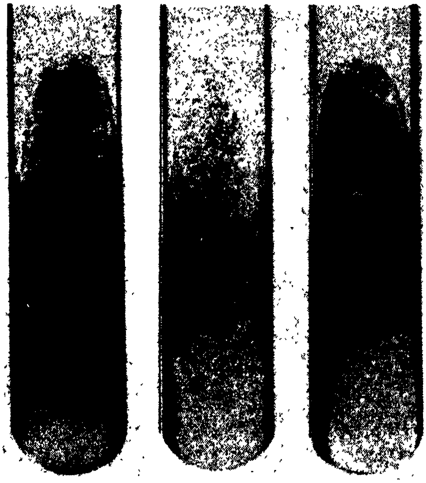

FIG. 1. *Penicillium funicolosum* grown 11 days on $NaNO_3$ agar. From left to right grown at 20° C. with no cultures of fungus B841; at 25° C. with cultures of fungus B841 in the cabinet; at 25° C. with no cultures of fungus B841.

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[3] The thiamine-peptone medium contained per liter of distilled water 0.5 g. $MgSO_4 \cdot 7H_2O$, 1.5 g. KH_2PO_4, 2 g. neopeptone, 0.5 ml. mineral supplements used in this laboratory, 50 g. dextrose and 1500 mμ moles thiamine.

[4] In making the $NaNO_3$ medium the peptone was replaced with an equal weight of $NaNO_3$. The pH of the agars was as follows: malt agar pH 4.9, thiamine-peptone agar pH 5.5, $NaNO_3$ agar pH 5.3.

of *Penicillium funiculosum* was substantially reduced in the cultures under the bell jar; the effect was most marked on the $NaNO_3$ medium (FIG. 2).

A series of microörganisms was tested in the same way. It included *Penicillium notatum, Ceratostomella ulmi, Merulius lachrymans, Poria incrassata, Phycomyces blakesleeanus, Agaricus campestris, Aspergillus niger, Rhizopus nigricans, Trametes pini, Rhodotorula aurantiaca, Saccharomyces cereviseae, Polyporus schweinitzii* and *Neurospora sitophila.*

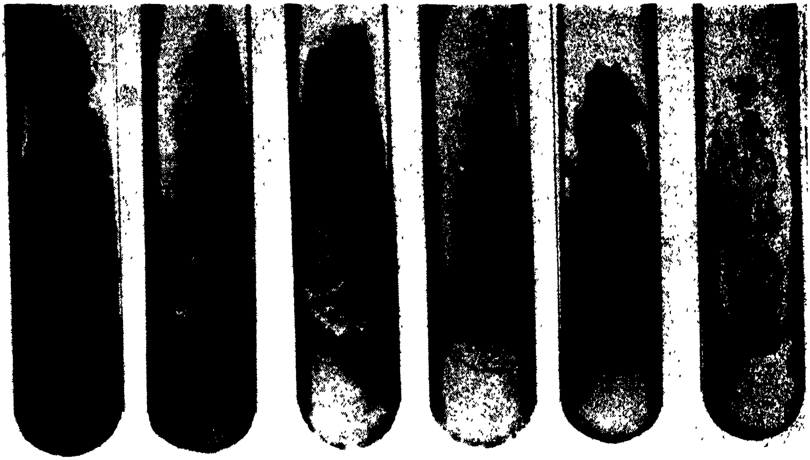

FIG. 2. *Penicillium funiculosum* grown at room temperature on various media under bell jar in presence of Fernbach flask containing fungus B841 and outside the bell jar. From left to right 11 days on malt agar outside bell jar; under bell jar; 14 days on $NaNO_3$ agar outside bell jar; under bell jar; 11 days on peptone agar outside bell jar; under bell jar.

All of the fungi tested showed some inhibition under the bell jar but the responses varied with the fungus and with the medium. Generally speaking, the inhibition was greater for growth on the $NaNO_3$ medium than for that on the malt or peptone media. Some of the organisms used grew very poorly on the $NaNO_3$ medium because of vitamin deficiencies or an unfavorable nitrogen source.

Aspergillus niger, Poria incrassata, Penicillium notatum, Polyporus schweinitzii, Neurospora sitophila and *Saccharomyces cereviseae* were affected least. At the end of 2 weeks there was little difference in the appearance of the cultures inside and outside the bell jar.

The mycelial growth of *Phycomyces blakesleeanus* and *Rhizopus nigricans* was substantial under the bell jars but the production of sporangiophores was materially reduced. On the malt and peptone agars *Phycomyces* formed sporangiophores in the cultures under the bell jar which failed to mature and both sporangiophores and mycelium were a deep yellow. *Rhizopus* developed few sporangiophores under the bell jar.

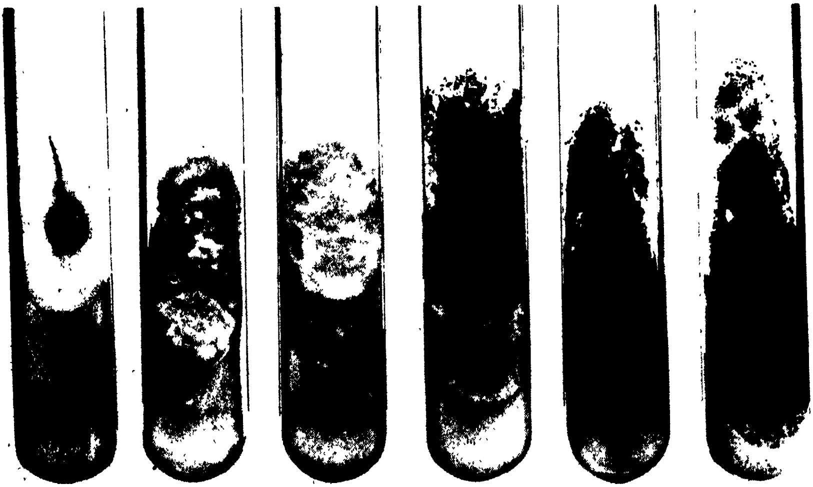

FIG. 3. Various fungi grown on thiamine-peptone agar at room temperature under bell jar in presence of Fernbach flask containing fungus B841 and outside the bell jar. From left to right *Merulius lachrymans* 26 days old under bell jar; outside bell jar; *Rhizopus nigricans* 20 days old under bell jar; outside bell jar; *Aspergillus niger* 20 days old under bell jar; outside bell jar.

The growth of *Ceratostomella ulmi, Penicillium funiculosum, Merulius lachrymans, Agaricus campestris, Trametes pini,* and *Rhodotorula aurantiaca* was markedly reduced. Typical responses are shown in figure 3.

Yellow pigment appeared in the agar of cultures of *Penicillium notatum* and *Merulius lachrymans* incubated outside the bell jars; pigment production was inhibited in cultures under the bell jar.

Tubes of sterile agar were incubated under a bell jar with a culture of fungus B841 for three days. The tubes were removed, inoculated with *Penicillium funiculosum*, and incubated in a cabinet containing no cultures of fungus B841. Comparable tubes which had stood outside the bell jar were inoculated and incubated at the same time. Growth was less in the tubes which had been under the bell jars for 3 days before inoculation. The effect was most marked for the $NaNO_3$ agar and least for the malt agar. This demonstrated that a volatile toxic substance produced by fungus B841 was absorbed by an agar medium incubated in the presence of a culture of the fungus under a bell jar.

The production of hydrocyanic acid by cultures of B841 was demonstrated by the Guignard test and by the Prussian blue method. Strips of filter paper impregnated with sodium picrate suspended in the air above the culture liquid of fungus B841 gave the characteristic color reaction for HCN. The contents of an open dish originally containing potassium hydroxide left in the incubator for several days gave a strong test for cyanide by the Prussian blue method. The odor of HCN was detected in the cabinet in which fungus B841 was incubated and in the bell jar which covered a culture of the fungus.

We were not successful in demonstrating the production of HCN by actively growing mycelium of fungus B841. It seems probable that the HCN originated from autolysis. Bach (1) found HCN in fruit bodies of *Pholiota aurea* (Matt.) and *Clitocybe geotropa* (Bull.) 48 hours after picking but none when they were fresh. The formation of the HCN appeared to be enzymatic and perhaps the result of the oxidative decomposition of amino acids.

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THE PHYSIOLOGY OF A BLUE STAIN MOLD WITH SPECIAL REFERENCE TO PRODUCTION OF ETHYL ACETATE[1]

MORRIS A. GORDON[2]

(WITH 9 FIGURES)

In the course of a survey of fungi causing blue stain of timber in Duke Forest, in the Spring of 1947, there was isolated from a blue-stained recently-cut stump of short-leaf pine (*Pinus echinata* Mill.) an ascomycete which produced, in culture, a strong ester odor, commonly described as banana oil odor, suggestive of the lower organic acetates. The present account is concerned with the identity of this fungus and isolation and identification of the odoriferous substance, with an attempt to discover the chemical mechanism of its formation.

Few accounts of ester production by fungi appear in the literature. Birkinshaw, Charles, and Raistrick (**4**) recovered ethyl acetate from cultures of *Penicillium digitatum* Saccardo. Birkinshaw and Findlay (**5**) identified methyl cinnamate, methyl p-methoxycinnamate, and an ester of anisic acid in cultures of *Lentinus lepideus* Fr., and Birkinshaw, Bracken, and Findlay (**3**) isolated methyl anisate from the metabolic products of *Trametes suaveolens* (Linn.) Fr. Nord and Vitucci (**12, 13**) found that *L. lepideus* produced methyl p-methoxycinnamate from various carbon sources, and attempted to elucidate the mechanism of its formation.

Several species of *Endoconidiophora* have been mentioned in connection with the formation of esters or of ester odors. Lagerberg, Lundberg, and Melin (**9**) reported "amyl acetate" production on various carbon sources by *Endoconidiophora coerulescens* Münch. According to Lagerberg (8) this was not established chemically, but only by odor. Morgan (11) found by chemical analysis that the odor produced by *E. coerulescens* Münch is caused largely by the presence of methylheptenone, although some iso-butyl acetate also was isolated. Davidson (6), in describing *E. moniliformis*, attributed to it an odor of banana oil and commented that "the production of a distinct odor seems to be a character common to all of the species . . . [of *Endoconidiophora*]". Davidson (7) states that *E. virescens* Davidson is characterized by a "musty penetrating odor" and *E. variospora* Davidson by an "odor similar to banana oil".

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MATERIALS AND METHODS

Isolation of the ascomycete from the stained wood was accomplished by implantation of perithecia-bearing splinters on 2.5 per cent Difco malt extract agar plates and transfer of ascospore masses from the resulting crop of perithecia to new plates. Stock cultures bearing always both perithecia and conidia were maintained on slants of the same medium in pyrex test tubes incubated at 20° C., and transfers made at 4–5 day intervals by mass inoculation of either ascospores or perithecia. By this procedure large concentrations of perithecia were produced with a minimum of accompanying mycelium and conidia. For the inoculation of liquid media, slants were flooded with 7–8 ml. of sterile distilled water and the resulting suspension of ascospores and conidia pipetted into the media in the indicated amounts. All cultures were incubated aerobically at 20° C. (± 1°) in the dark, except as otherwise noted.

Media were sterilized at 15 lbs. for 15–20 minutes, except in the case of urea and certain other indicated heat-labile components which were filtered through a Seitz filter and added aseptically. Thiamin was autoclaved separately.

In growth measurements, where "no growth" is noted, more or less extensive germination has occurred in each case. "Inhibition" indicates lack of germination.

Dry weights were determined by filtration of liquid cultures (25 ml. of medium) through tared oven-dried Whatman No. 1 filter paper in Gooch crucibles and drying of the fungus mats for 48 hours in a hot-air oven maintained at 100–105° C.

Ester titrations were done according to A.O.A.C. (1) procedure, on cultures grown in 250 ml. Erlenmeyer flasks containing 100 ml. of medium. In each case the mat was broken up with dissecting needles and the entire contents of the flask distilled.

Hydrogen ion concentrations were measured with a Beckman pH meter, necessary adjustments of the media being made with NaOH and H_2SO_4.

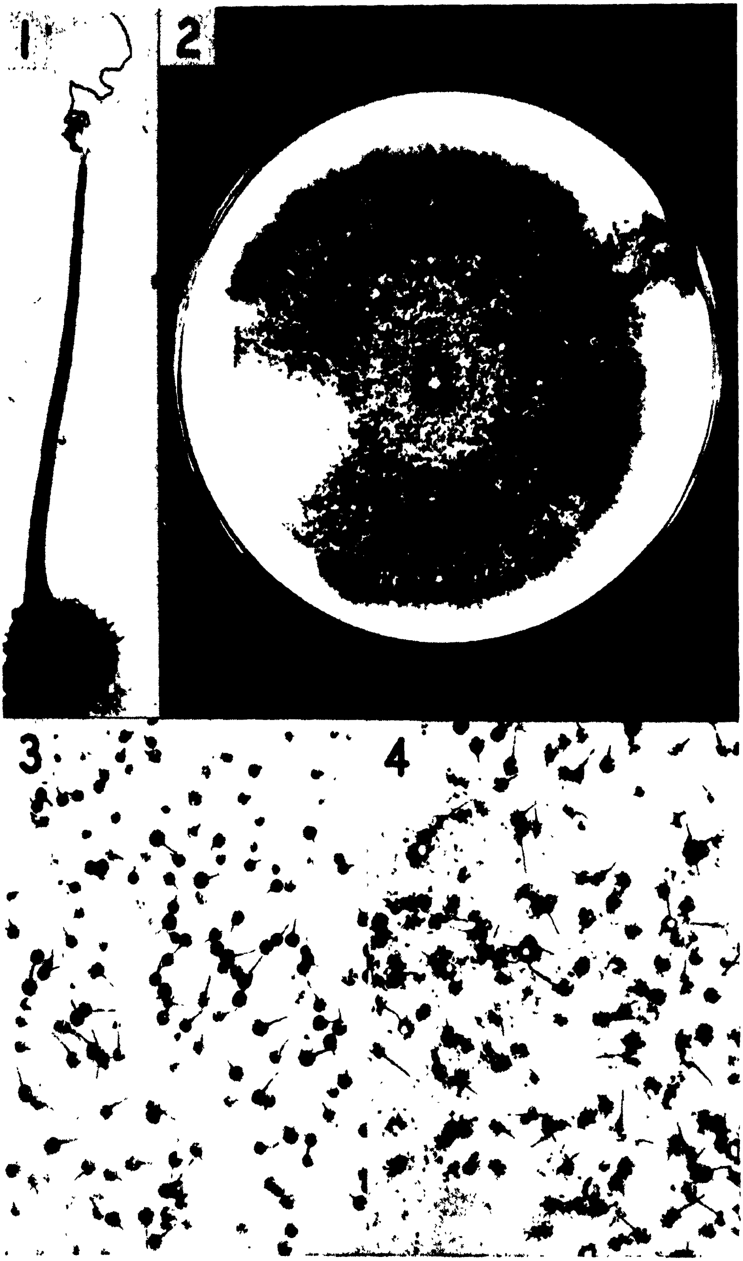
All chemicals were of C.P. or reagent grade unless otherwise specified.

Glassware was cleaned by treatment with dichromate-sulfuric acid solution and rinsed with tap and distilled water.

Further details of method for separate phases of the work will appear in the appropriate ensuing sections.

EXPERIMENTAL RESULTS

Identity of the wood-staining organism: The mold concerned produces the typical long-beaked carbonaceous perithecia (FIG. 1) characteristic of the family Ceratostomataceae. In figure 2 are illustrated the abundant perithecia obtained when this mold was cultured on malt agar. In such cultures the white mycelium becomes darker with age, turning tan and finally dark brown. As is shown in figures 3 and 4, the bulbous base of the perithecium is formed first and, as this matures, the elongate beak grows out therefrom. The beak may be oriented perpendicularly but more often lies at various angles to the substrate, frequently parallel to it. The perithecial base is studded with dark brown to black spines (FIG. 5). Ascus walls are either evanescent or entirely absent, for the ascospores exude single-file through the beak (FIG. 6), pushing aside the fimbriate apical fringe and remaining agglutinated, in either air or an aqueous mounting medium, by virtue of their gelatinous capsules. The spores are ovoid in shape but when unstained have a derby-hat appearance on account of the peculiar capsule. The delimitation of this outer coat is apparent in figure 7, showing spores which have been differentially stained with cotton blue.



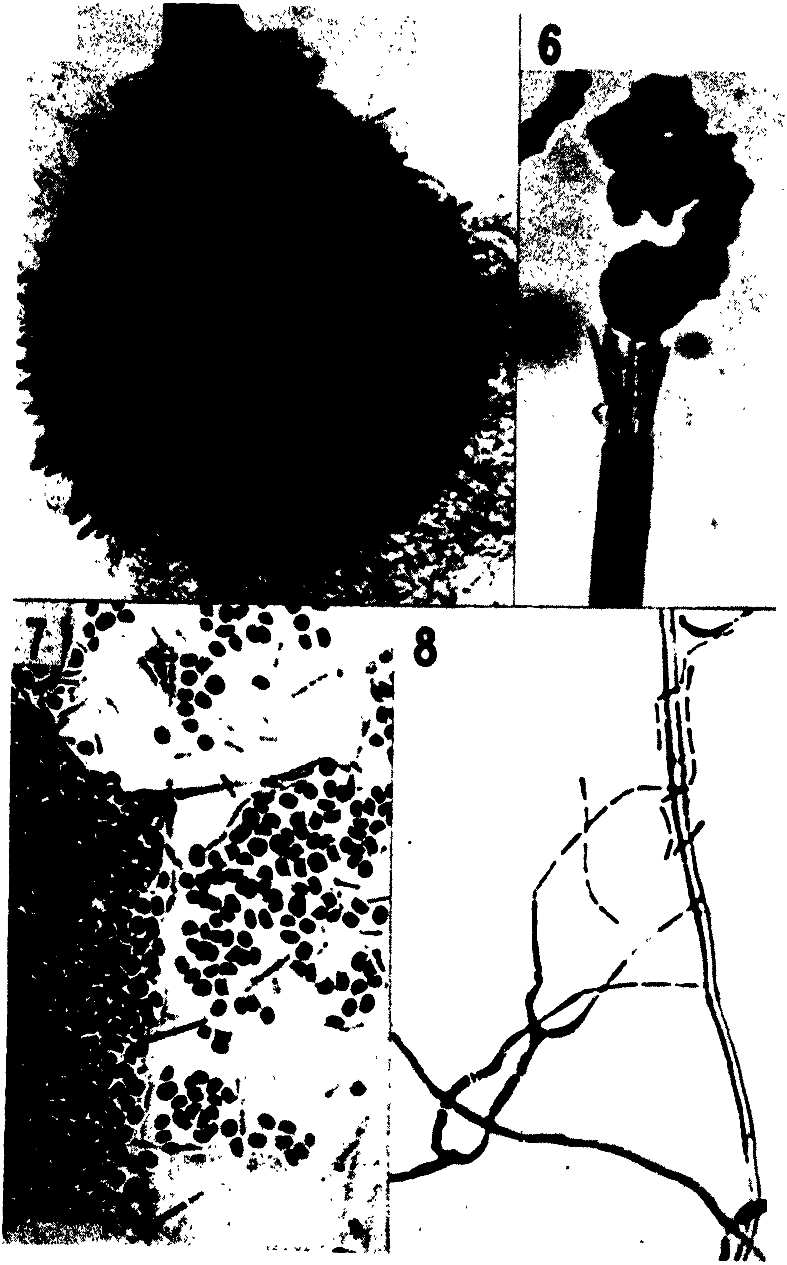
FIGS. 1–4. Blue stain fungi.

The imperfect stage of this mold is distinctive in that conidia are formed endogenously and extruded seriatim from the conidiophore in long moniliform chains (FIG. 8). The appearance of the conidial stage, combined with the characteristics enumerated above, defines this organism as *Endoconidiophora moniliformis* (Hedgc.) Davidson.

For purposes of comparison, a subculture of the organism studied by Davidson and used by him in describing *E. moniliformis* was obtained from Baarn, Holland. This culture was devoid of perithecia, but the two cultures were identical with respect to mycelial growth, type of conidiophore, and size and shape of endoconidia. Although two kinds of endoconidiophores had been described for *E. moniliformis*, only one, the long attenuated variety, appeared in subcultures of the Baarn specimen and in this respect it agreed entirely with the new isolate. When grown on 2.5 per cent Difco malt extract agar, each yielded narrow, hyaline, cylindric conidia which subsequently enlarged, many becoming barrel-shaped and eventually yeast-shaped. Finally, each culture produced a "banana-oil" odor indistinguishable from that of the other.

Basic conditions for growth of E. moniliformis and production of the ester odor: E. moniliformis made good growth, including production of both conidial and perithecial stages, on malt agar, Sabouraud's agar, nutrient agar, carrot-sucrose agar and potato-dextrose agar, when incubated at room temperature (20–25° C.) with no attempt at control of illumination. There was little mycelial growth on corn meal agar, but a few perithecia were produced. The characteristic odor was produced on all of these media.

The following chemically defined medium, essentially that employed by Robbins and Ma (**14**) in their study of the vitamin requirements of certain species of *Ceratostomella*, was, when supplied with 0.4 micromole of thiamin (GBI thiamin hydrochloride) per liter, found to compare very favorably with malt extract media in ability to promote growth, production of perithecia, and formation of the characteristic odor by *E. moniliformis:* dextrose 50 g., KH_2PO_4 1.5 g., $MgSO_4 \cdot 7H_2O$ 0.5 g., asparagin 2.0 g., H_2O q.s. 1000 ml., plus the following trace elements in parts per million: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Mn, 0.01 Mo, and 0.09 Zn. This



FIGS. 5–8. Blue stain fungi.

solution, thiamin included, will be referred to hereinafter as the "basal medium."

It was found in preliminary experiments that this mold is not deficient for either biotin or pyridoxine.

The optimum temperature for diameter growth of the present isolate of *E. moniliformis* on both malt agar and the basal medium solidified with agar is approximately 25° C. At this temperature, the average colony diameter on the former medium was 77 mm. and on the latter 85 mm., after 4 days. Amount of odor production appeared to be correlated with diameter growth at each temperature. At 20° C. growth was only slightly less than the optimum and, since a constant-temperature room at 20° was available, all subsequent cultures were incubated at this temperature.

E. moniliformis grows well over a wide range of hydrogen-ion concentrations, producing the ester odor at concentrations between pH 4.22 and pH 6.66 on various synthetic media. All media herein employed were adjusted to pH 4.5–5.0, except as otherwise indicated, this being about the range of pH for different batches of the unadjusted basal medium.

Relation of ester production to growth with respect to time: In order to follow quantitatively the course of ester production and to fix the time of its maximum occurrence in these media, a number of flasks of both malt extract solution (2.5 per cent) and the basal medium, in 100 ml. quantities, were prepared and each inoculated with 0.5 ml. of spore suspension. At frequent intervals, usually of 2–4 days, one flask of each medium was harvested and titrated for total esters (expressed as ethyl acetate; 1 ml. of 0.1 N alkali = 0.0088 g. ethyl acetate). Parallel with this, a time-growth series was run on both media, in which dry weight of mycelium and pH of filtrate were determined for serial samples.

Results are represented by a graph in figure 9, where values for pH and dry weight represent the average for triplicate samples inoculated with 0.1 ml. of spore suspension and grown on 25 ml. of medium in 125 ml. flasks. It is seen that maximum ester production on the basal medium occurs at about 12 days and is closely correlated with maximum dry weight. This suggests that after the original carbon source is exhausted the fungus utilizes the ester which it has produced. There appears to be no such simple relationship on the more complex malt medium.

Nitrogen requirements of E. moniliformis with respect to growth and ester production: A series of experiments designed to elicit some clue as to the mechanism of formation of the ester was undertaken. Lampitt (10) showed that microorganisms (*Saccharomyces cerevisiae*) could deaminate asparagin with the formation of ethyl alcohol. Since this transformation might be involved in the present chemical sequence, it was thought desirable to vary the nitrogen source in the basal medium in order to determine whether asparagin is essential to the manufacture of the ester. If asparagin could be eliminated in favor of a simpler nitrogen

TABLE 1

COMPARATIVE GROWTH, QUALITY OF ESTER ODOR AND QUANTITY OF TITRATABLE ESTERS PRODUCED BY *E. moniliformis* WHEN SUPPLIED WITH VARIOUS NITROGEN SOURCES IN A BASAL MEDIUM CONTAINING 5.0 PER CENT DEXTROSE AS A CARBON SOURCE

Nitrogen compound in g./l.		[a] Comparative growth	[a] Quality of ester odor	[b] Mg. ester per 50 ml. of medium
Control—no added nitrogen		No growth	- -	
Inorganic:				
KNO_3	3.00	++	None	
KNO_2 [c]	2.55	Inhibition	—	
NH_4NO_3	1.20	++	Very weak	
$(NH_4)_2SO_4$	1.98	++	Fair	
$(NH_4)_2C_2O_4 + H_2O$	1.86	+++	Good	
$(NH_4)_6Mo_7O_{24} + 4H_2O$	1.85	Inhibition	—	
$NH_2OH \cdot HCl$	2.08	Inhibition	—	
Amides:				
Asparagin	1.98	++++	Good	62.8–18 days
Urea [c]	0.90	++++	Good	89.3–12 days
Amino acids:				
Glutamic acid, Merck	4.41	+++	Good	
Aminoacetic acid, Merck	2.25	++++	Fair	
B-Alanine, Merck	2.67	+	None	
dl-Alanine, Eastman	2.67	++++	Good	
l (-) Leucine, Eastman	3.93	++++	Good	56.3–18 days
l-Cystine, Eastman [d]		+	None	
l-Tyrosine [d]		++++	Fair	
Bactopeptone	2.60	++++	Good	
Malt extract	2.70	++++	Fair	

[a] After 14 days; average of 2–3 flasks.

[b] Each determination on one flask; expressed as ethyl acetate; No. of days as noted.

[c] These compounds sterilized separately by filtration.

[d] Dissolved to limit of solubility.

compound, then glucose could be regarded as the only initial material from which the ester was derived.

Seventeen compounds, containing nitrogen in various valence forms, were substituted individually in equimolar nitrogen quantities for asparagin in the basal medium. In addition, a control set with asparagin and one with only atmospheric nitrogen available were employed. Each solution was prepared in triplicate 100 ml.

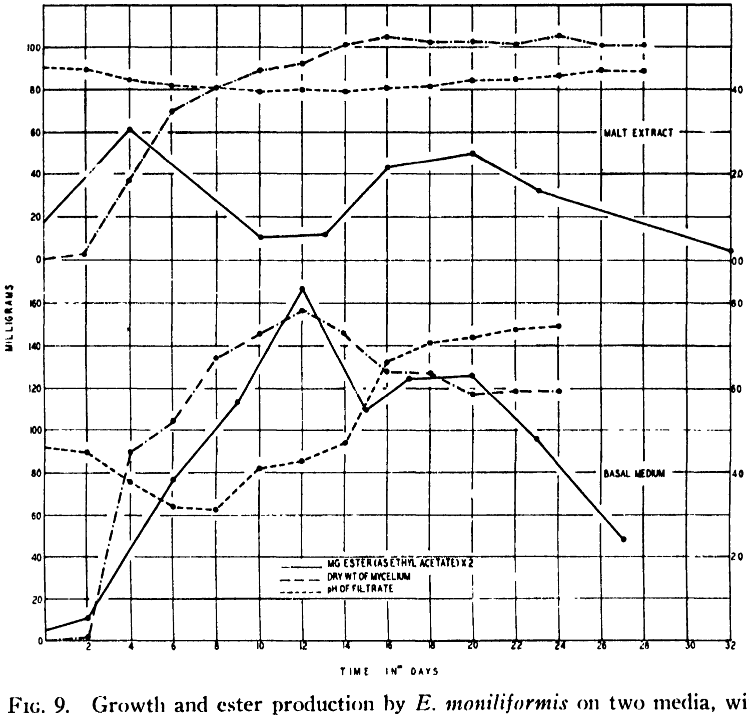


FIG. 9. Growth and ester production by *E. moniliformis* on two media, with corresponding changes in pH of the solutions.

quantities distributed to 250 ml. flasks and 0.5 ml. of spore suspension was used as inoculum. Table 1 shows comparative growth, odor production and, in a few instances where the odor was considered particularly promising, ester titrations for these media.

The inorganic nitrogen compounds employed permitted little or no growth of *E. moniliformis* under the conditions of this experiment, three of them, potassium nitrite, ammonium molybdate, and hydroxylamine hydrochloride, preventing germination when added in the stated amounts. (In preliminary experiments, conidia and ascospores of this organism germinated readily in distilled water.) Of the organic nitrogen sources tested, urea, peptone, malt extract, and several amino acids permitted mycelial production comparable to that obtained when asparagin was incorporated as the nitrogen compound. Urea, furthermore, appeared to be at least the equal of asparagin in permitting production of the ester and had the additional advantage, for use in the further study of the phase-sequence concerned, that it is a relatively simple substance and one not likely to be involved directly in the synthesis.

TABLE 2

COMPARATIVE GROWTH, QUALITY OF ESTER ODOR AND QUANTITY OF TITRATABLE ESTERS PRODUCED BY *E. moniliformis* WHEN SUPPLIED WITH VARIOUS CARBON SOURCES IN A BASAL MEDIUM CONTAINING 0.09 PER CENT UREA AS A NITROGEN SOURCE

Carbon compound in g./l.		[a] Comparative growth	[a] Quality of ester odor	[b] Mg. ester per 50 ml. of medium
Control—no added carbon		No growth	—	
Soluble starch	45.0	++++	Good	
Cellobiose	50.0	++++	Fair	16.2–13 days
Dextrose (pH 4.39)	50.0	++++	Excellent	88.6–11 days
Mannose	50.0	++++	Good	74.6–9 days
Galactose	50.0	++++	Doubtful	15.0–13 days
Arabinose	50.0	++++	None	
Xylose	50.0	+++	None	
Glycerol	25.0	+	None	
Calcium lactate	50.0	No growth	—	
Pyruvic acid [c,d] (pH 5.38)	4.0	++	None	
Pyruvic acid[c,d]	1.0	++	None	
Acetaldehyde[c,e] (pH 5.18)	5.0	Inhibition	—	
Acetaldehyde[c,e]	1.0	No growth	—	
Ethyl Alcohol [c]	10.0	++++	Good	72.4–15 days
Ethyl Alcohol [c]	5.0	++++	Fair	
Acetic acid [f]	10.0	Inhibition	—	
Ethyl alcohol [c] + Acetic acid[f] (1:1)	10.0	Inhibition	—	
Ethyl alcohol [c] + Acetic acid [f] (1:1)	5.0	+++	Good	
Ethyl acetate [c]	2.0	++	None	

[a] Estimated after 14 days.

[b] Each determination on one flask; expressed as ethyl acetate; No. of days as noted.

[c] These compounds sterilized separately by filtration.

[d] Added as sodium pyruvate, prepared from Pyruvic Acid, Eastman.

[e] Freshly distilled from Paraldehyde, Eastman.

[f] Added as sodium acetate.

Carbon requirements of E. moniliformis with respect to growth and ester production: With urea (0.9 g. per l.) as the nitrogen source, various carbon compounds were substituted for glucose in the basal medium in an effort to clarify further the mechanism of ester formation. 100 ml. of media were distributed in triplicate to 250 ml. flasks and inoculated with 0.5 ml. of spore suspension. The results are presented in table 2. Values for growth and for quality of odor represent the average for at least 2 flasks.

Soluble starch, the disaccharide cellobiose, and the hexoses, mannose and galactose, supported good growth of *E. moniliformis*, approximately equal to that on media containing dextrose, but only with starch and mannose, of these compounds, was the ester odor considered good. The 2 pentoses, arabinose and xylose, yielded good to fair mycelial production, but no trace of the ester aroma. Of the 3-carbon compounds employed which are commonly involved in anaerobic respiration and alcoholic fermentation, glycerol and pyruvic acid permitted little growth and no acetate odor, while no growth was observed with lactic acid as the carbon source. Acetaldehyde did not support growth when employed at a concentration of 0.1 per cent, and 0.5 per cent of this compound inhibited germination. Ethyl alcohol, however, served as a good carbon source for production of both mycelial mat and ester, although the yield of both was either impaired or completely prevented by the addition of acetic acid to the alcohol medium. Acetic acid itself, in a concentration of 1.0 per cent, inhibited germination of the spores. Ethyl acetate, 0.2 per cent, when used as a sole carbon source, permitted a small amount of mycelial growth.

Isolation and identification of ethyl acetate: 1) From culture on malt extract: In an attempt to isolate and characterize the substances responsible for the banana oil odor, 102 one-liter flasks, each containing 350 ml. of 2 per cent malt extract solution, were inoculated with 2.0 ml. of spore suspension per flask. The initial pH of this medium was 4.58. Several flasks were harvested each day from the fourth day following inoculation until the twelfth day, when the last batch was processed, each group of cultures being filtered through cheesecloth and the fungus mat and filter cloth stored in a refrigerator. The filtrate was saturated with sodium chloride and extracted with 4 portions of ether. These were then combined, washed with a portion of 10 per cent Na_2CO_3 followed by several portions of distilled water saturated with NaCl, and finally with 50 per cent $CaCl_2$ solution, then dried over anhydrous $MgSO_4$. The collected mats and filter cloths were steam-distilled and 4 liters of distillate were subjected to ether extraction as above. The dried ether extractives were combined and most of the ether evaporated off through a long fractionating column. The residue was then distilled from a small flask and the following fractions obtained:

1) 34–73° C.: ca. 4.0 ml.
2) 73–82° C.: ca. 0.5 ml; odor like ethyl acetate.
3) 83–107° C.: ca. 0.5 ml.; odor like ethyl acetate.
4) Residue: 0.5 ml.

The first 3 fractions were recombined and fractionated through a semi-micro vigreux column, yielding the following new fractions.

a) 36–60° C.: ca. 2.0 ml.
b) 70–77° C. (Mainly 77°): ca. 0.5 ml.; odor of ethyl acetate.
c) Residue: less than 0.5 ml.

Fraction b) (0.4086 g.) was hydrolyzed by boiling with an excess of N/1 alcoholic NaOH for 1½ hours under reflux. Excess NaOH was titrated with 0.25 N HCl to aqueous phenolphthalein. Saponification equivalent = 96.92. The neutralized solution was distilled and the distillate gave a positive iodoform test at 60° C. (not in the cold).

Thus the odorous substance is probably ethyl acetate.

Residues 4) and c) were combined and subjected to the test for fusel oil as described by Basset (2). The result was negative. Preliminary control runs showed that this test is negative for methyl and ethyl alcohols in concentrations of 1 : 100, and positive for n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, n-amyl, iso-amyl, and tert-amyl alcohols at the same concentration. N-amyl acetate at a concentration of 1 : 5000 gave a positive test. The higher-boiling fractions therefore contained no significant quantity of acetates of the 3-to-5-carbon alcohols.

2) From culture on the basal medium: A similar recovery procedure was followed with 104 flasks, each containing 350 ml. of the basal medium. All flasks were harvested after 12 days and the mats, instead of being steam-distilled, were ground up and extracted directly. After removal of the ether, a total of 3 ml. of distillate was collected in the range 70–78° C. This distillate had a well-defined ethyl acetate odor, but no further purification or characterization of the fraction was attempted.

A more efficient method for recovering the ester was provided by the use of vacuum distillation. Materials for use in vacuum distillation were secured by the following procedure: Loosely wadded cotton balls, 3–4 cm. in diameter, were soaked in the basal medium and excess liquid was expressed by light pressure of the fingers. Seven one-liter flasks were half-filled with these cotton balls and autoclaved. For each flask, 2 malt agar slant cultures were flooded with sterile distilled water, the spore suspensions poured into 50 ml. of basal medium and the mixture distributed aseptically over the flask contents. Incubation was at room temperature (20–25° C.) for 7–10 days. One flask was inoculated per day for 7 successive days and the cultures were harvested serially. As the contents of each flask matured (detected by odor), they were transferred to a vacuum oven connected in series with 2 dry ice-alcohol traps and a high-vacuum pump. Suction was applied continuously for 8 hours and the vacuum maintained overnight. In two instances during this run, several ml. of distillate reached the second trap, and in each case appeared there in 2 layers. The upper layer in each case was separated, the 2 combined, washed with 50 per cent $CaCl_2$ and dried over anhydrous Na_2SO_4. The liquid was then poured off into a side-arm flask and distilled, practically the entire contents (1.5 ml.) coming over at 71–81° C. (fraction 1a).

The remainder of the vacuum distillate from the entire run was extracted with several portions of ether, the ether solution being then washed with 50 per cent $CaCl_2$ and dried over anhydrous

Na_2SO_4. Following evaporation of most of the ether, the residue was distilled and the following fractions collected:

1b) 33–65° C.: ca. 2 ml.
2b) 65–80° C. (mainly 77–80° C.): ca. 2 ml.

Fractions 1a) and 2b) were combined and refractionated through a semi-micro vigreux column, yielding the following new fractions:

1c) 32–74° C.: ca. 1.5 ml.
2c) 74–77.5° C. (mainly 77.1°): ca. 2 ml.; odor of ethyl acetate.

0.3658 g. of fraction 2c) was hydrolyzed by boiling with excess N/1 NaOH for 65 minutes under reflux. Excess NaOH was titrated with 0.1 N HCl to aqueous phenolphthalein. Saponification equivalent = 101.9. The neutralized solution was distilled and the distillate gave a positive iodoform test when warmed to 60° C. Further determinations on fraction 2c): micro boiling-point 77.0° C.; refractive index at 18.9° = 1.3722 (ethyl acetate = 1.37216. Handbook of Physics and Chemistry, 28th Edition).

3) From culture on basal medium modified by replacement of carbon and nitrogen sources: In order to establish the identity of the ester produced in the dextrose-urea and alcohol-urea media, the following procedures were employed. Seven liters of the former medium were prepared and distributed in 350 ml. amounts to 20 one-liter flasks. Initial pH was 4.22. Each flask was inoculated with 2.0 ml. of spore suspension and incubated at 20° C. for 13 days. The entire contents of the flasks were then blended in a Waring Blendor, and the resulting mixture distilled through an 18-inch fractionating column. Two fractions were collected:

1) 70–97° C.: 30 ml. consisting of 2 layers.
2) 97–98.2° C.: 80 ml. consisting of 2 layers.

The upper layers were drawn off and combined (total 6.5 ml.), shaken with an equal volume of 50 per cent $CaCl_2$ solution, separated, and dried over anhydrous $MgSO_4$. The remainder of the collected distillate was then refractionated and 3 fractions obtained:

1a) 69–73° C.: upper layer of 6 ml., water insoluble; lower layer (aqueous), less than 0.5 ml.

2a) 76.5–78.5° C. (mainly 78°): 4 ml. aqueous.
3a) 80.0–99.0° C. (gradual rise): 5 ml. aqueous.

Fraction 1a) was shaken with an equal volume of 50 per cent $CaCl_2$ solution, separated, and dried over anhydrous $MgSO_4$. It was then combined with the upper layers of fractions 1) and 2) and distilled from a small Spitz-type fractionating apparatus, practically the entire volume coming over in the range 70.0–79.8° C. This was collected in four parts:

a) 70.0–74.9° C.: 4 ml.; odor like ethyl acetate.
b) 75.0–76.9° C.: 3 ml.: odor of ethyl acetate.
c) 77.0–77.1° C.: 1 ml.: odor of ethyl acetate.
d) 77.5–79.8° C.: ca. 2 ml.; odor like ethyl acetate.

0.2010 g. of fraction c) was hydrolyzed by boiling with an excess of N/1 alcoholic NaOH for 2 hours under reflux. Excess NaOH was titrated with 0.25 N HCl to aqueous phenolphthalein. Saponification equivalent = 97.6.

A portion of fraction b) was subjected to an elementary analysis by the sodium fusion method (Shriner and Fuson, **15**) and gave negative tests for nitrogen, sulfur and halogens. A second portion was treated with 3,5-dinitrobenzoyl chloride and sulfuric acid and the resulting 3,5-dinitrobenzoate recrystallized from ethanol. The crystals melted at 92.9° C. (ethyl ester of 3,5-dinitrobenzoic acid = 93.0° C.). A third portion was refluxed with hydrazine hydrate and the hydrazide recrystallized from a water-ethanol mixture. The purified substance melted at 66.9° C. (hydrazide of acetic acid = 67° C.). The production of ethyl acetate is thus confirmed.

Fractions 2a) and 3a) were shown to contain ethyl alcohol by the following procedure. Two ml. of the liquid were treated with 3,5-dinitrobenzoyl chloride and the resulting precipitate recrystallized from a water-ethanol mixture. Melting point = 93.0° C.

Ten one-liter flasks, each containing 350 ml. of the alcohol-urea solution, were inoculated with 2.0 ml. of spore suspension each, incubated at 20° C. and harvested after 15 days. Treatment was the same as that described for the dextrose-urea medium, resulting in the recovery of 6 ml. of distillate in the range 70.0–80.0° C. S.E. on 75.0–77.1° fraction = 95.0. Elementary analysis negative for nitrogen, sulfur and halogens. Odor of ethyl acetate. M.P. of 3,5-dinitrobenzoate = 92.8° C. M.P. of hydrazide = 66.9° C. Ethyl acetate is therefore produced from ethyl alcohol as a carbon source, in the presence of urea as a nitrogen source.

DISCUSSION

On media in which ethyl acetate is formed, the amount produced apparently parallels the growth of the mycelium. This, together with the fact that *E. moniliformis* can utilize ethyl acetate as a sole carbon source, indicates that the ester is utilized only after more readily available nutrients are exhausted. Acetic acid is apparently toxic to the organism, even preventing germination when employed as the carbon source in a synthetic medium. Its esterification by ethyl alcohol manifestly serves as a mechanism whereby the toxicity of the acid is removed. It would appear that the oxidation of ethyl alcohol to acetic acid and subsequent enzymatic coupling of these two compounds comprise the final steps in the phase-sequence of ethyl acetate production. Since acetaldehyde in relatively low concentrations supports no growth and even inhibits germination, its possible role here as an intermediary substance in the manufacture of ethyl acetate must be considered doubtful. Lactic acid, pyruvic acid, and glycerol, substances which are involved in ordinary anaerobic fermentation, also permit little or no growth of *E. moniliformis*. Xylose and arabinose, although serving well as carbon sources for mycelial production, yielded no detectable odor of ethyl acetate, whence it may be assumed that little or none was produced. It is probable, therefore, that no significant amount of alcohol was generated from these sugars, although *Fusarium lini* Bolley was shown by White and Willaman (**16**, **17**) to produce ethyl alcohol from pentoses as well as from hexoses and apparently via similar pathways.

SUMMARY

1. A mold causing a blue stain of pine wood, identified as *Endoconidiophora moniliformis* (Hedgc.) Davidson, grows well in a solution of mineral salts, dextrose, and asparagin, when supplied with thiamin. It has a wide range of pH tolerance on this and

other media, and an optimum temperature for mycelial growth of approximately 25° C.

2. Considerable quantities of ethyl acetate as well as some ethyl alcohol are produced in the above solution, and the ester is formed also in malt extract solution, but no higher acetates were detected in cultures on either medium.

3. The production of esters on the dextrose-asparagin medium is correlated closely with gain in dry weight by the fungus, a maximum for both being attained in about 12 days at a temperature of 20° C., following which the esters are gradually decomposed.

4. *E. moniliformis* produces good growth, accompanied by varying amounts of ester, on the above medium when asparagin is replaced by any of several organic nitrogen compounds, notably urea, but in general neither growth nor ester production is pronounced when inorganic nitrogen sources are used. The dextrose-urea medium yields relatively large quantities of ethyl acetate.

5. When urea is employed as the nitrogen source and other carbon compounds substituted for dextrose in the medium, it is found that soluble starch, mannose, cellobiose, and galactose permit good growth and good to poor ester production, and 2 pentoses good growth but no ester odor. Various 3-carbon intermediates in the usual chain of alcoholic fermentation support little or no growth and yield no detectable ester odor. Acetic acid in relatively low concentrations inhibits germination and growth of the organism, but ethyl acetate is utilized for the production of mycelium.

Ethyl alcohol serves as an excellent carbon source for *E. moniliformis* with respect to both growth and production of ethyl acetate.

6. The significance of these findings for the elucidation of the phase-sequence of ethyl acetate synthesis by *E. moniliformis* is discussed.

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EXPLANATION OF FIGURES

FIG. 1. Perithecium extruding ascospores. Stained with cotton blue (× 92).

FIG. 2. Malt agar plate culture: numerous perithecia; white mycelial growth on periphery (× 4/5).

FIG. 3. Malt agar plate culture: stages in development of perithecia (× 8).

FIG. 4. Malt agar plate culture: mature perithecia with apical clumps of ascospores (× 8).

FIG. 5. Base of perithecium: numerous spines (× 256).

FIG. 6. Mouth of perithecium: ascospores being extruded. Stained with cotton blue (× 404).

FIG. 7. Mass of ascospores, showing gelatinous capsules. Stained with cotton blue (× 556).

FIG. 8. Conidiophores and endoconidia. Stained with cotton blue (× 556).

NOTES ON TWO LITTLE KNOWN BIRD'S NEST FUNGI FROM SOUTHERN UNITED STATES

HAROLD J. BRODIE

(WITH 2 FIGURES)

As was pointed out by Lloyd, there are but four common and widely distributed species of the Nidulariaceae in the United States. Lloyd (3) reported that of one hundred and seventy-six specimens he had received from correspondents, one hundred and sixty-five belonged to one or other of the following: *Crucibulum vulgare* Tul., *Cyathus stercoreus* (Schw.) De Toni, *C. striatus* Willd., *C. vernicosus* DC.

The purpose of this note is to draw attention to two species from southern United States, one of which appears to be scantily represented in the herbaria of the southern states, the other to be known within the United States from only a single collection. It is believed that these species might be more frequently collected if their characteristics were better known and both are therefore illustrated and described in this paper.

CYATHUS POEPPIGII Tul.

In Miss White's monograph (4) this *Cyathus* was reported from the Danish West Indies. It was originally described from Cuba. Lloyd (3) lists collections from Mauritius, German East Africa, Australia and Samoa. Coker and Couch (1) refer to the first collection of this species in the United States from Gainesville, Florida, 1924. Some months ago, the writer examined some specimens of *C. Poeppigii* collected by Dr. Erdman West at Gainesville, Jan. 16, 1941. Dr. L. R. Hesler, who kindly loaned the specimens from the Herbarium of the University of Tennessee, was of the opinion that this species had also been found in Tennessee.

West's collection consists of some two dozen fruit bodies that had evidently developed in clusters. The cups are *dark chocolate* *brown,* about 8 mm. high and 5–6 mm. wide at the mouth. Superficially they might be mistaken for small depauperate forms of *C. striatus. C. Poeppigii* is distinguished macroscopically by the striations or grooves on the upper one-third of the *outside* of the cups (FIG. 1). However, because the young fruit bodies are hirsute, the external striation is apt to be overlooked. Moreover, the writer has examined more than one collection of *C. striatus* in which there is slight striation on the outside of the cup. One such collection from Indiana was actually labelled "*C. Poeppigii*" by the collector. There is *no tunica* covering the mature peridioles of *C. Poeppigii* and these therefore appear very dark brown or black and shiny (FIG. 1). The basidiospores are *very large,* measuring 22–25 × 30–32 μ in the West collection. The spore size, of course, distinguishes this species very readily from *C. striatus.* A comparison of the characteristics of *C. Poeppigii* and *C. striatus* is given in table 1.

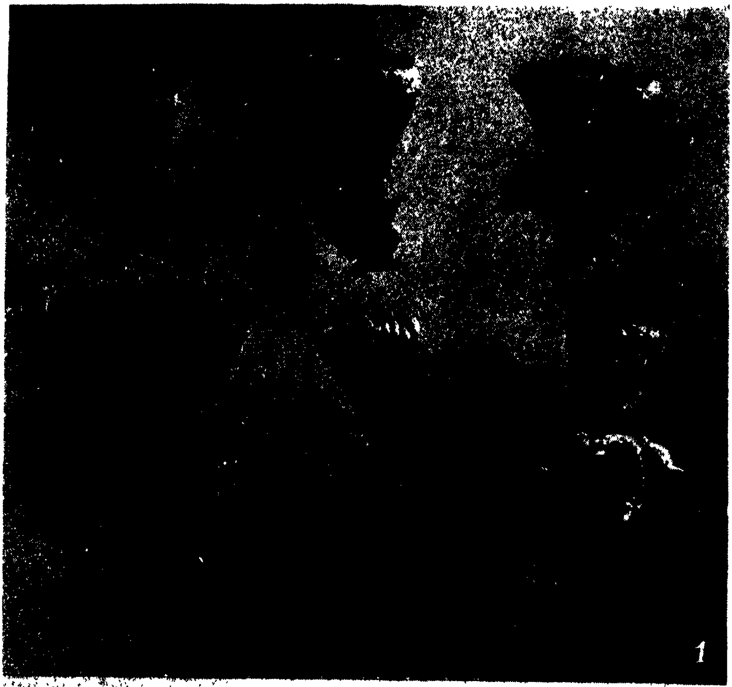
TABLE 1

COMPARISON OF *Cyathus Poeppigii* AND *C. striatus*

	Cyathus Poeppigii	*Cyathus striatus*
Size	8–10 mm. high; 5–6 mm. wide at mouth.	8–12 mm. high; 6–8 mm. wide at mouth.
Shape	Broad—conic.	Usually more elongate, conic.
Color	Chocolate brown.	Light to medium brown although darker forms occur.
Outer Surface	Shaggy or hirsute; hairs around mouth inconspicuous.	Very hirsute; hairs around mouth usually very conspicuous.
Striation	Marked *external* striation as well as internal.	Striae *internal*; if external, very inconspicuous.
Peridioles	Lacking tunica; dark brown to black; 1.5–2 mm. in diameter.	Usually with evident light-colored tunica
Basidiospores	Large, variable; mostly 22–25 × 30–35 μ.	Small, variable; mostly 8–12 × 18–20 μ.

CYATHUS PALLIDUS Berk. and Curt.

As far as the writer has been able to ascertain, this species has been collected in the United States only once. It was found by



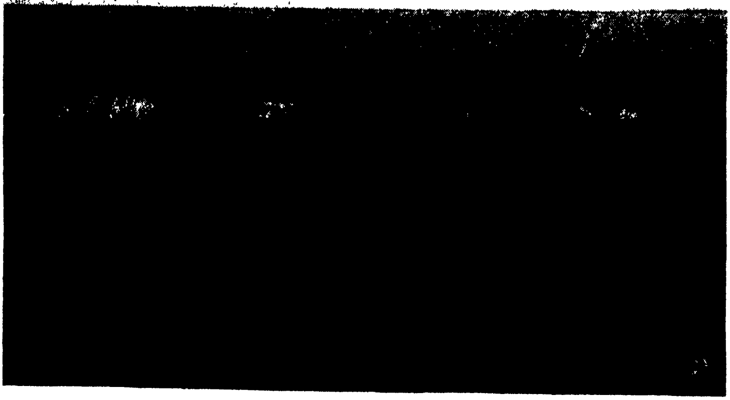


FIG. 1. *Cyathus Poeppigii* fruit bodies and peridioles. The grooves or striae on the outside of the peridium and the dark shiny peridioles devoid of tunica are characteristic of this species. Specimens from a collection made by Dr. Erdman West, Gainesville, Fla., Jan. 16, 1941. × 2.

FIG. 2. *Cyathus pallidus* fruit bodies and peridioles. The two specimens on the left show the coarse hairs characteristic of this species. Specimens collected by Dr. William Diehl, Savannah, Ga., Jan. 6, 1933. ×1⅛.

Dr. William Diehl (Jan. 6, 1933) at Savannah, Georgia, and reported by him in Mycologia, 1941 (2). The specimens were growing on decaying boxes that had formerly contained cuttings of camellia plants. The species was originally described from Cuba and was reported by Lloyd (3) from Jamaica and Antigua and by Miss White (4) from Cuba.

Dr. Diehl kindly allowed the writer to examine his collection of *C. pallidus* and the following notes are based upon this examination. The four specimens of this fungus (FIG. 2) are *pale buff* or fawn-colored, 7–9 mm. high and 8–9 mm. wide at the mouth. In shape they are widely conical. The peridium is *thin* and membranous and is covered externally with hairs. In addition there are *coarser rigid hairs* that are quite conspicuous. In Dr. Diehl's specimens, the cups are very faintly ridged on the inner surface. Lloyd (3) commented: "The type specimens appear *very slightly striate,* but the plant belongs in the section Olla, not in Eucyathus as found in Saccardo."[1] The peridioles are 2 mm. in diameter and have a *thin tunica.* Basidiospores are *small,* mostly 7 × 8.5 μ in the Diehl specimens, thick-walled and colorless.

TABLE 2

COMPARISON OF *Cyathus pallidus* AND *C. stercoreus*

	Cyathus pallidus	*Cyathus stercoreus*
Size	7–9 mm. high; 8–9 mm. wide at mouth.	Variable, from 5–10 mm. high; strains as small as *C. pallidus* occur.
Shape	Broad—conic.	Variable but usually with sides less divergent than *C. pallidus.*
Color	Pale gray-brown.	Brown or gray.
Outer Surface	Hispid with numerous rigid hairs.	Very shaggy with matted hairs.
Peridioles	With delicate tunica; 2 mm. in diameter.	Devoid of tunica; dark brown or black; 2 mm. in diameter.
Basidiospores	Small; subglobose to elliptical; 5–7 × 8–10 μ.	Large; subglobose; 30–35 μ.

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[1] The italics are Lloyd's.

Because this species is not unlike several collections from Florida of a small gray form of *C. stercoreus* (except for the large spores of the latter) which the writer has seen, a comparison of the two species is given herewith (table 2).

The superficial resemblance of *Cyathus Poeppigii* to small dark forms of *C. striatus,* and of *C. pallidus* to small pale forms of *C. stercoreus* may have resulted in the two rare species being overlooked by collectors. The appearance of these fungi in Georgia and Florida suggests their introduction from the adjacent tropics. Dr. Diehl (2) found *C. pallidus* growing underneath camellia cuttings in a commercial nursery and Dr. West found *C. Poeppigii* at Gainesville, Fla.

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NOTES AND BRIEF ARTICLES

ANNUAL FORAY, 1948

Inasmuch as the A.A.A.S. had announced plans for an autumn meeting for 1948, it was decided to hold the annual foray of the Society in the early summer. Such a course had two advantages, namely, there would not be two meetings within a short space of time, and an opportunity would be afforded to collect representatives of the late spring and early summer flora.

Through the kindness of Dr. A. H. Stockard, the director of the University of Michigan Biological Station, the Society was enabled to utilize the facilities of the Station from June 15 to 17. The Station is located in Cheboygan County in the northern part of the southern peninsula of Michigan.

On Tuesday morning, June 15, collections were made in the Carp Creek gorge, an area with a primarily coniferous cover. Identification of material and further collecting at nearby sites occupied the afternoon. In the evening, before a most welcome open fire in the recreation hall, members were treated to an extraordinarily fine display of Kodachrome pictures of fungi by E. B. Mains, A. H. Smith and B. B. Kanouse, of the University Herbarium. Wednesday, June 16, was devoted to an all day trip to the scenic Harbor-Springs-Cross Village area bordering Lake Michigan. Collections were made in the hardwoods bordering the lake. After a picnic lunch, collections were made at other points along the return route to the Biological Station. That evening, Dr. E. B. Mains explained and illustrated a series of stereokodachrome pictures of fleshy fungi which is in preparation. Thursday, June 17, was devoted to collecting in two nearby bogs. In the morning the group went to Mud Lake Bog where a number of interesting finds were made. In the afternoon Reese's Bog, bordering Burt Lake, was the collecting area.

Although an early summer drought did not make collecting as fruitful as it might have been, a goodly number of interesting speci-

Annual foray, 1948, Douglas Lake, Michigan.

mens were brought back to the Station. A list of these together with their collectors is appended.

I would be remiss, indeed, if special mention were not made at this time of the yoeman service performed by A. H. Smith in making the foray a success. His familiarity with the terrain was of tremendous value. Nor were Smith's contributions to the foray confined entirely to fleshy fungi. It was with pardonable pride that the Vice-President noted he was evidently educating certain well-known rust and agaric specialists in the methods of finding aquatic fungi. At least, so it seemed, for every evening at dusk these gentlemen would adjourn to some nearby stream with boots and straw collecting baskets. Since the substrata obtained never seemed to possess saprolegniaceous fungi on them, they were promptly utilized for other worthy but distinctly non-mycological purposes.

The following is a list of the fungi collected. The collector's names are abbreviated and in parentheses, *i.e.*, St. = Stevenson, S. = Sinden, Ri. = Reischer, etc.

LIST OF SPECIES

Achlya flagellata: 4 collections from Maple River, June 15, 1948. 2 from Lake Shore cut off, June 16, 1948. 1 from Mud Lake Bog, June 17, 1948 (Ri.)

Achlya Klebsiana (probably): 1 collection from Maple River, June 15, 1948 (Ri.)

Achlya papillosa: 2 collections from Mud Lake Bog, June 17, 1948 (Ri.)

Achlya polyandra (probably): 1 collection from Maple River, June 15, 1948 (Ri.)

Ascochyta cassandrae Pk., on *Chamaedaphne calyculata* (L.) Moench. (S.)

Bifusella Faullii Darker, on *Abies balsamea* (L.) Mill. (S.)

Chrysomyxa cassandrae (P. & C.) Tranz., on *Chamaedaphne calyculata,* Mud Lake Bog, June 17, 1948, Rogerson & JAS. (St.)

Chrysomyxa cassandrae (P. & C.) Tranz., on *Chamaedaphne calyculata* (L.) Moench. (S.)

Chrysomyxa chiogenis Diet., on *Chiogenes hispidula* (L.) T. & G. (S.)

Chrysomyxa ledi (A. & S.) de Bary, on *Ledum groenlandicum* Oeder (S.)

Chrysomyxa ledi (Alb. & Schw.) de By, on *Ledum groenlandicum,* Mud Lake Bog, June 16, 1948 (St.)

Daedalea unicolor Bull. ex Fr., on *Betula* (dead wood), below Cross Village (St.)

Erysiphe graminis D. C., on *Agropyron repens,* Grounds, Biol. Sta., June 15, 1948 (St.)

Fomes pini (Fr.) Karst., on *Picea glauca,* Mud Lake Bog, June 17, 1948, det. J. L. Lowe (St.)

Fomes pinicola (Fr.) Cke., on *Tsuga canadensis,* Gorge, Biol. Sta. U. Mich., June 15, 1948 (St.)

Fomes scutellatus (Schw.) Cke., on *Alnus* (dead twigs), Mud Lake Bog, June 17, 1948 (St.)

Fomes subroseus (Weir) Overh., on *Abies balsamea,* Mud Lake Bog, June 17, 1948 (St.)

Herpobasidium filicinum (Rostr.) Lind., on *Dryopteris disjuncta* (Rupr.) Morton (S.)

Hyalopsora aspidiotus (Pk.) Magn., on *Abies balsamea,* above Harbor Springs, June 16, 1948, Kern et al. on *Phegopteris dryopteris* above Harbor Springs, June 16, 1948, Kern et al. (St.)

Hyalopsora aspidiotus (Pk.) Magn., on *Abies balsamea* (L.) Mill. and on *Dryopteris disjuncta* (Rupr.) Morton (S.)

Hymenochaete tabacina (Sow. ex Lév.), on *Alnus* (dead branches), Mud Lake Bog, June 17, 1948 (St.)

Hypodermella punctata Darker, on needles of *Abies balsamea,* below Cross Village, June 16, 1948; on needles of *Abies balsamea,* Mud Lake Bog, June 17, 1948 (St.)

Irpex cinnamomeus Fr., on *Quercus* (dead branches)—above Harbor Springs, June 16, 1948 (St.)

Isoachlya unispora: 1 collection from Carp Creek, the Gorge, June 15, 1948. 1 from Maple River, June 15, 1948 (Ri.)

Lachnum virgineum (Batsch. ex Fr.) Karst., on dead twigs, Mud Lake Bog, June 17, 1948 (St.)

Leptolegnia caudata: 1 culture from Mud Lake Bog, June 17, 1948 (Ri.)

Leptothyrium periclymeni (Desm.) Sacc. var. *americanum* E. & E., on *Lonicera canadensis* Marsh (S.)

Mycosphaerella chimaphilina (Pk.) House, on *Chimaphila umbellata,* below Cross Village, June 16, 1948 (St.)

Mycosphaerella chimaphilina (Sacc.) House, on *Chimaphila umbellata* (L.) Bart. var. *cisatlantica* Blake (S.)

Peltigera canina (L.) Willd., Reese's Bog, June 17, 1948 (St.)

Peniophora cinerea Fr., on *Betula* (dead twigs), Gorge, Biol. Sta., June 15, 1948 (St.)

Phyllachora Witrockii (Erikss.) Sacc., on *Linnaea borealis* L. var. *americana* (Forbes) Rehder (S.)

Polyporus abietinus Dicks. ex Fr., *Tsuga canadensis,* Gorge, Biol. Sta., June 15, 1948; *Betula* sp., Gorge, Biol. Sta., June 15, 1948 (St.)

Polyporus biformis Kl., on dead wood, below Cross Village, June 16, 1948 (St.)

Puccinia amphigena Diet., on *Calamovilfa longifolia,* above Harbor Springs, Emmet Co., June 16, 1948 (St.)

Puccinia caricis-grossulariata Arth., on *Grossularia,* Maple River, June 15, 1948 (St.)

Puccinia coronata Cda., on *Rhamnus alnifolia,* Reese's Bog, June 17, 1948 (St.)

Puccinia coronata Cda., on *Rhamnus cathartica* L. (S.)

Puccinia extensicola solidaginis (Schw.) Arth., on *Solidago altissimum,* Reese's Bog, June 17, 1948; on *Solidago* sp., Reese's Bog, June 17, 1948 (St.)

Puccinia pringsheimiana Kleb., on *Ribes* sp. (S.)

Puccinia violae (Schum.) D. C. (?), on *Viola* sp., Gorge, Biol. Sta., June 15, 1948 (St.)

Pucciniastrum Pyrolae (Pers.) Schroet., on *Pyrola secunda* L. (S.)

Ramularia arvensis Sacc., on *Potentilla norvegica* L. var. *hirsuta* (Michx.) Lehm. (S.)

Ramularia Magnusiana (Sacc.) Lindau, on *Trientalis borealis* Raf. (S.)

Rhytisma andromedae (Pers.) Fr., on *Andromeda glaucophylla* Lk. (S.)

Saprolegnia ferax: 2 collections from Carp Creek, the Gorge, June 15, 1948 (Ri.)

Saprolegnia litoralis: 2 collections from Carp Creek, the Gorge, June 15, 1948 (Ri.)

Septoria coptidis Berk. & Curt., on *Coptis trifolia,* Mud Lake Bog, June 17, 1948 (St.)

Septoria osmorrhizae Pk., on *Osmorrhiza* sp., Maple River, June 15, 1948, J. C. Gilman (St.)

Septoria trillii Pk., on *Trillium,* above Harbor Springs, June 16, 1948 (St.)

Septoria trillii Pk., on *Trillium grandiflorum* Michx. (S.)

Steccherinum ochraceum (Fr.) S. F. Gray, on *Alnus* (dead bark), Mud Lake Bog, June 17, 1948 (St.)

Stereum fasciatum Schw., on *Betula* (fallen log), below Cross Village, June 16, 1948 (St.)

Stereum sanguinolentum (Alb. & Schw.), on *Abies balsamea,* below Cross Village, June 16, 1948 (St.)

Taphrina ?bacteriosperma Johans., on *Betula papyrifera* Marsh var. *cordifolia* (Regel) Fern. (S.)

Taphrina caerulescens (Mont. & Desm.) Tul., on *Quercus ?borealis* Michx. F. (S.)

Taphrina caerulescens (Mont. & Desm.) Tul., on *Quercus maxima,* Biol. Sta., June 15, 1948 (St.)

Taphrina dearnessii Jenkins, on *Acer rubrum* L. (S.)

Taphrina dearnessii Jenkins, on *Acer rubrum,* Reese's Bog, June 17, 1948, on *Acer rubrum,* also seen at Biol. Sta., June 15, 1948 (St.)

Taphrina flava Farl., on *Betula papyrifera,* Reese's Bog, June 17, 1948 (St.)

Taphrina virginica Seym. Sadeb., on *Ostrya virginiana* (Mill.) Willd. (S.)

Thraustotheca clavata: 1 culture from Mud Lake Bog, June 17, 1948 (Ri.)

Uromyces acuminatus Arth., on *Smilacina stellata,* above Harbor Springs, June 16, 1948 (St.)

Uromyces acuminatus Arth., on *Maianthemum canadense,* above Harbor Springs (Emmet C.), June 16, 1948 (St.)

Uromyces caladii (Schw.) Farl., on *Arisaema triphyllum,* Maple River, June 15, 1948 (St.)

Uromyces caladii (Schw.) Farl., on *Arisaema atrorubens* (Ait.) Blume. (S.)

Venturia dickiei (Berk. & Br.) Ces. & de N., on *Linnaea borealis* L. var. *americana* (Forbes) Rehder (S.)

Venturia dickiei (Berk. & Br.) Ces. & de Not., on *Linnaea borealis,* Reese's Bog, June 17, 1948 (St.)

Venturia pulchella C. & P., on *Chamaedaphne calyculata* (L.) Moench. (S.)

—F. K. SPARROW.

SHOULD DR. D. P. ROGERS' PROPOSALS FOR THE REGULATION FOR FIXING THE GENERIC TYPES BE ACCEPTED AS PUBLISHED IN MYCOLOGIA 41: 219, 1949?

The proposals made by Dr. D. P. Rogers regarding the fixation of the generic types are extremely important for further taxonomic work in the fungi as well as in other special fields of botany. It is the writer's impression that they should be discussed widely before they are submitted to the International Congress at Stockholm. As a member of the "Special Committee on Fungi for the International Congress at Stockholm," the writer is expected to either approve or disapprove of proposals submitted, but in the case of Dr. Rogers' proposals, a mere yes or no would not be sufficient. On one hand, this proposal has the merit to give the taxonomist a "clear, simple, and honest" (as Rogers says himself) tool for the selection of types; on the other hand it is too rigid and explicit to satisfy those who insist on keeping all additional rules in line with the basic idea as expressed in Art. 3 (1) "to aim at fixity of names" and Art. 18, Recommendation VI, "choose a species that will fix the generic name as it is now commonly applied." For example, following the proposed rules as edited by Rogers one would have to exclude the generic name *Cantharellus* and either abandon (as a synonym) or change the sense of the genus *Psathyrella.* Rogers Art. 5(b) would, if interpreted literally, imply that only species with white spores are typical for *Cantharellus* since this is stated in the original description. The typical *Cantharelli* in the sense of all authors have colored (yellow or rosy) spores while those species originally admitted in *Cantharellus* that have white spores, are now excluded from the genus. However, being forced by a rigid rule, one would have to exclude the species generally accepted (Earle, Murrill, Clements & Shear, Singer & Smith, Doty) as generic type, *Cantharellus cibarius,* and admit one of the white-spored species instead which would upset the taxonomy of the whole complex. In the case of *Psathyrella,* one would have to follow the even more dangerous rule indicated in Art. 6 ("Among species equally eligible, the preference shall be given to the first known to have been designated as the type"); this would be *Psathyrella disseminata,* now separated from *Psathyrella* in the sense of all modern authors and either incorporated into *Coprinus,* or type species of a small genus, *Pseudocoprinus.* Consequently, *Psathyrella* would become a synonym of *Coprinus* or it would replace *Pseudocoprinus,* and the species of *Psathyrella,* now more numerous than ever, would have to be transferred to *Hypholoma,* or *Psilocybe,* both used in another sense by some mycologists, and in the sense of *Psathyrella* by none. These examples might be supplemented by others of the same group of fungi as well as by examples from other cryptogams and phanerogams. One can by no means imply that these or similar generic names belong to what Rogers calls "a beloved, but illegitimate nomenclature." In the contrary, the acceptance of the rules proposed by Rogers would tend to "cause error and ambiguity." Rogers' proposals would have been excellent if they had been published 30 years ago. Now, they will help solving some of the problems accumulated because of the absence of such rules, but at the same time, they will destroy that partial structure of sound, acceptable generic names created by the common sense of many authors and tradition of up to two centuries. Aside from that, one will have to consider the difficulty of establishing definitely the first designation of a type species, and the changes in what is considered disagreement with an original diagnosis.

The writer proposes to use Rogers' proposal (or a similar one) as a basis but restricting the rules to articles 1–3, or possibly 1–5(a), referring Art. 4–6, or at least 5b, and 6 to the recommendations, since such a set of rules and recommendations does not and should not solve every single problem of typification. However, the writer cannot see why the remaining difficulties cannot be overcome by lists of "Species Lectotypicae" such as given in the supplement written by Hitchcock & Green for the Linnean generic names of the Phanerogamae. This has the additional advantage to promote the preparation of lists by specialists in various groups thus avoiding the rigid application of rules which would upset the nomenclature in certain cases. After such lists have been submitted, they should be discussed, and judged by the sincerity with which the author has applied the existing rules and recommendations, and by the respect he has shown for the names based on established custom, as long as they are legitimate.

Whether Appendix I in the end takes the form of Rogers' proposals, or as here proposed, that of a short and simple set of rules and recommendations plus a supplement of lists of "Species Lectotypicae"—let us not forget that action one way or the other is preferable to rejection of or inaction on both proposals.

—ROLF SINGER.

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UNIVERSIDAD NACIONAL DE TUCUMÁN

THE MYXOMYCETES [1]

The embodiment of Dr. G. W. Martin's long-continued studies in the taxonomy of the Myxomycetes has recently appeared as volume 1, part 1, of North American Flora. Students of these fungi will note a considerable change from the arrangement of the component groups appearing in Macbride, Macbride & Martin, and Hagelstein, the predecessors of the present work. In addition, a number of small genera and of poorly-marked species have been combined with related groups with the purpose of better expressing the existing relationships.

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[1] MARTIN, G. W. Fungi. Myxomycetes. Ceratiomyxales, Liceales, Trichiales, Stemonitales, Physarales. North American Flora 1 (1): 1–151. Bibliography, by H. W. Rickett, pp. 153–178. Index, pp. 179–190. 1949—New York Botanical Garden, $7.25.

Although parts of the North American Flora dealing with fungi have been appearing since 1906, it has been left to Dr. Martin to define (on p. 1) the Division *Fungi*.—DONALD P. ROGERS.

Notice to Contributors to Mycologia

Professor G. W. Martin of the Department of Botany of the University of Iowa has been elected to the position of Editor-in-Chief of Mycologia for the five year term beginning with the volume for 1951. After September 1, 1950 all manuscripts should be submitted to him at the above address. Professor Martin expects to be in Europe for some months previous to the above date, so the present incumbent will function until that time. This is important to those who will be submitting papers for the last two issues of 1950 since if such papers are sent to Professor Martin during the summer months they might accidentally be neglected until work is started on the 1951 issue.—ALEXANDER H. SMITH.

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FUNGI IN RELATION TO THE DEGRADATION OF WOOLEN FABRICS[1]

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(WITH 7 FIGURES)

INTRODUCTION

The purpose of this investigation was to test the ability of a few common saprophytic molds and a series of human pathogenes to degrade woolen fabrics, and further, to elucidate generally the question of fungous degradation of such materials.

The interest of members of the Quartermaster Laboratories, which had hitherto been primarily in cellulosic materials, was extended, beginning late in 1945, to include studies on the microbiological degradation of woolen fabrics. At that time a report was received from the Office of the Quartermaster General that troops in moving from one location to another were sometimes required to pack woolen clothing hastily into barracks bags, and upon reaching the destination would find the garments molded and in a disagreeable condition. Someone had made a preliminary survey of the molded fabrics and had named *Fusarium moniliforme* Sheld. as the species mainly responsible.

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[1] Presented in part at the A.A.A.S. Meetings, Chicago, Dec. 1947.

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The original source of the report and the geographical area in which the annoyance occurred were never learned, and no great amount of faith was placed in the scientific accuracy of the determination of *F. moniliforme* as the causal organism. However, no stretch of the imagination is required to think of such clothing—perhaps damp, dirty, and sweaty—becoming molded when placed in bags in damp climates. The organisms involved, though seriously objectionable, need not necessarily be actual wool degraders.

The microbiological deterioration of woolen fabrics in its fundamental and more purely scientific aspects is part of a broader problem having to do with the nutritional relationship of microorganisms to keratinous materials in general—wool, hair, nails, horn, hoof, feathers, etc. Our knowledge of organisms capable of growing on such materials has been slowly expanded, dating from the observations of *Onygena equina* on old hoofs and horns in the very early days of the history of mycology to the very recent records contained in a series of papers by Dr. John S. Karling. Karling has successfully used various keratinous materials to trap members of the Chytridiales from soil and aquatic habitats. Studies on physiological and chemical aspects of the utilization of keratins by the dermatophytes have recently been reported by Page (**1948**), and a program of fundamental research (Mandels, Stahl & Levinson, **1948**; Stahl, McQue & Siu, **1948**) has been in progress at the Quartermaster Laboratories since shortly after the inception of the work, in 1945, on which the present paper is based.

Turning more specifically to the question of the fungous degradation of woolen fabrics, to which the present paper will be strictly confined, the information available tends to be rather scanty and not very specific. The degradation of "felts" in the paper industry has long been a problem, but the cause presumably is wholly or for the most part bacterial. It is a matter of common observation among those individuals who are engaged in research on deterioration problems that woolen fabrics when subjected to standard soil-burial procedures will decay as readily as do cotton fabrics subjected to similar tests. During the war the number of complaints about the molding of woolen goods was minor compared with the number concerned with the molding of cotton products. To some extent this is due to the fact that in regions where mold problems are greatest, there is relatively little wool used. On the other hand there is no doubt but that fewer fungi are capable of attacking wool than are capable of attacking cotton. Some cases of molded wool were reported, however, and some samples of such material did reach the writers. There still remains the problem of making detailed studies and tests of some of the fungi that were isolated from such materials.

In 1946 one of the present authors had the opportunity of making a field survey of the degradation of materials around the tropical belt of the world. The more concentrated attention was on cellulosic materials but some incidental observations were also made on the behavior of wool. Samples were returned which exhibited severe degradation by Actinomycetes and at the same time showed resistance to attack by fungi. Caps, field jackets, trousers, etc., made up of combinations of cotton and woolen materials often showed severe molding of the cotton parts, whereas the adjacent woolen portions had remained free from attack. Photographic illustrations of such molded items, accompanied by brief observational data, have already been presented (White, **1946**a, b; White, Siu & Reese, **1948**, p. 628–629).

LITERATURE REVIEW

Those who have worked on the problem of the molding of woolen fabrics from the point of view of the textile industry have not drawn a very clear-cut distinction between molds utilizing finishing materials and those utilizing the substance of the wool itself. It is evident that mold in any case is objectionable. Burgess appears to have been the first worker to enter the field. In a series of papers extending over a ten-year period beginning in 1924 (those concerned with fungi being cited in the bibliography) he dealt primarily with the molding of fabrics in storage and transit. He stressed the role of agents used in the manufacture of woolen goods in rendering the fabric susceptible to molding in storage. These were such materials as soaps, oils, and conditioning fluids. While some substances modified the fiber itself to make parts of it utilizable, it appeared that most of the increased susceptibility was due to the nutritive value of the processing or finishing material. The mildewing properties of both scoured and unscoured wool were greatly reduced by thorough washing with water. Though the action of heat in rendering wool susceptible to attack was not stressed by Burgess, it had become well known even at that time that wool was subject to modification by the action of heat. Burgess (**1928**) did, however, call attention to the difficulty of testing fungi on wool because of the partial hydrolysis and resulting increased susceptibility brought about by discontinuous steaming which was the method of sterilization that he employed. But, for the most part, those interested in the microbiology of wool gave little consideration to the effect of ordinary laboratory sterilization in rendering wool susceptible to attack by organisms. Humfeld, Elmquist, & Kettering (**1937**) demonstrated fully that standard autoclaving, as well as other heat and various chemical treatments, modified the chemical and physical properties of wool. Their interest was chiefly in sterilization to prevent transmission of human bacterial diseases. Perhaps there was inference that the modification was in the direction of increased susceptibility to bacterial degradation, but no work was done with fungi.

The question of fungi versus bacteria as degraders of woolen goods has been given some attention, with general agreement (Burgess; Thaysen, Bunker, Butlin & Williams, **1939**; Harold, **1941**; Shanor, **1945**; Berk, **1947**) having been reached on certain important phases of the problem, *viz:* that woolen fabrics are ordinarily less susceptible to attack by microorganisms than cotton goods; that wool is none-the-less subject to attack by both fungi and bacteria; that wool is not likely to be attacked unless the humidity is very high, considerably above that necessary for attack on cotton; that any annoyances in damp storage are likely to be due to fungi, a state of wetness or near wetness being necessary for bacterial action.

Several workers have studied the relation of fungous pathogens of the human skin to woolen clothing and have called attention to the possibility of the transmission of skin diseases through woolens. However, cases of actual transmission have been difficult to prove conclusively, and it is still largely presumptive that woolen clothing is more apt to transmit skin diseases than cotton or any other kind of clothing that may be transferred from contact with diseased to normal skin. Gould and Carter (**1931**) inoculated small squares of a sweated woolen athletic jersey with the ringworm fungus, *Tricho-*

phyton interdigitale, and reported that the fungus remained viable for some months, as was also true of inoculum placed on glass, but that it did not grow on the wool. Their conclusion was that pathogenic fungi might live on and be transmitted by woolen clothing but were not likely to grow there. Bonar and Dreyer (**1932**) investigated the growth and transmission of fungi through the medium of clothing, laundries, the wood and debris of dressing room floors, etc. From this work it appears that ringworm fungi may be transmitted through infested skin scales in clothing, either wool or cotton, that they are capable of growth on, and decomposition of, woolen but not cotton fabrics. The temperatures used in standard power laundry practice for white cottons were sufficient to kill the fungi, but that employed for woolens and colored fabrics was doubtful. Dry cleaning solvents had negligible action on the fungi. The fungi tested were *Trichophyton interdigitale, T. rosaceum, Epidermophyton cruris,* and *Microsporon lanosum.* Berberian (**1938**) placed woolen, cotton, and silk stockings in separate Erlenmeyer flasks with a small amount of water, autoclaved them, and inoculated them with spores of *Trichophyton interdigitale.* He reported that at the end of three or four weeks there was marked growth on all of them. He also reported growth on several other materials. Emphasizing the role of footwear in transmission and reinfection he recommended formaldehyde sterilization of such materials. Rogers, Hirschmann and Humfeld (**1940**) found *Trichophyton interdigitale* capable of destroying squares of autoclaved, undyed, laundered, wool blanket material when they were placed on water agar, mineral salts agar or nutrient agars and inoculated with a spore suspension. They emphasized the practical importance of their findings both in the transmission of the athlete's foot disease and in the probable role of the fungus in the utilization of the fabric, thus shortening the period of serviceability. On the basis of the exploratory work reported in the present paper the ringworm fungus *Microsporum gypseum* appeared to be the most suitable of the fungi for biochemical studies on wool degradation. The strain (*PQMD 196*) on which later work was based (Mandels, Stahl, Levinson, **1948**; Stahl, McQue, & Siu, **1948**; Stahl, McQue, Mandels, & Siu, **1948**) was isolated by Mary Downing on September 12, 1946 from a strip of olive-drab woolen cloth that had been removed July 31 after having been subjected to indoor soil burial at the Philadelphia Quartermaster Depot for one week. It will be noted that strains received later from Dr. Emmons were perhaps equally vigorous.

The role of the common air and soil borne molds in relation to woolen materials is very vague, though several workers have given incidental attention to the identity of organisms isolated from or growing on wool. Burgess (**1924**) emphasized the role of bacteria, stated that it was common knowledge that molds also grow on wool, but obtained mostly negative results when molds were placed on sterile (presumably interval steaming) commercially scoured wool. Eventually he did find a few strains which fruited vigorously —*Aspergillus niger,* an undetermined *Aspergillus* and a *Penicillium.* Among fungi found growing naturally on wool he mentioned *Dematium* (possibly = *Pullularia pullulans*), other Fungi Imperfecti; and yeasts. The same author (**1928**) continuing pure culture tests called attention to the difficulty and uncertainty of such tests due to the hydrolysis caused by the discontinuous steaming. On wool thus treated he obtained growth of *Fusarium* (2 spp.), *Cephalothecium roseum, Aspergillus niger, A. fumigatus, A.* (2 spp.), *Penicillium brevicaule, P.* (3 spp.), *Acrostalagmus* sp., and *Trichoderma* sp., but furnished no quantitive data of any kind. Prindle (**1935**) studied the microflora of samples of raw back and shoulder wool from Western U. S. He found large numbers of saprophytic bacteria and molds which he assumed to represent contamination from soil, water, and air. Molds of the genera *Stemphylium, Penicillium, Oöspora, Alternaria,* and *Mucor* were isolated. Most of them were washed away or inactivated in the process of a scouring and drying. Some isolates from all of the foregoing genera except *Mucor* were claimed to cause complete destruction of fiber in worsted yarn, some causing discoloration without destruction. Hirschmann, Zametkin, and Rogers (**1944**) found degreased, steam-sterilized wool to support growth of *Bacillus mesentericus, B. subtilis,* and *Actinomyces albus* in the presence of added nutrients. The one fungus with which they worked, *Chaetomium globosum,* was able to grow on wool in the presence of only distilled water. The fiber was relatively resistant to all four organisms. *C. globosum* was less active in fiber disintegration than *A. albus* which was the strongest of the lot.

Karling, to whom reference was made earlier, has started a series of papers (**1946**; **1947**) on keratinophilic chytrids, indicating that ability to utilize keratin, or possibly even restriction to keratinous materials, may be not uncommon among the Chytridiales. Two species, *Rhizophydium keratinophilum* (**1946**) and *Phlyctorhiza variabilis* (**1947**), both new, were trapped on keratinous substances and found to grow on all common forms of keratin-containing tissues, including wool, but not on other materials. In so far as is known they are strictly soil inhabitants, presumably existing there on bits of keratinous substances, and are of no economic importance in the destruction of fabrics in use or storage.

EXPERIMENTS

Experiment 1. Action of nine common molds on steam sterilized wool serge (table 1).

Nine molds commonly isolated from cotton fabrics after exposure in the tropics, and of known cellulolytic activity, were selected.

Strips of 18-oz., olive-drab, wool serge were prepared by cutting to a size slightly larger than 6 × 1 in. and raveling to exactly that size. These were placed one each in 200 × 25 mm. test tubes each containing 25 ml. of mineral salts (formula A, Greathouse *et al.*, **1942**) medium so that approximately the lower half of each strip was submerged. The tubes were then plugged with cotton and *autoclaved for 20 minutes at 15 lbs. pressure.* Inoculum was made up by agitating sterile distilled water on potato dextrose agar slants on which the fungi were in good sporulating condition. Two ml. of the resulting spore suspension were distributed over the above-liquid portion of each strip by means of a pipette. Incubation of the strips was at 85° F. Following incubation the strips were removed, washed first in 1 : 1000 mercuric chloride, rinsed in water, dried on paper towels in the laboratory atmosphere, subjected to standard conditioning, and broken on the motor-driven Scott tester. Fungal activity was measured in terms of decline in breaking strength.

The results are presented in table 1.

The following points may be made by way of summary and comment.

1. Of 9 organisms tested on wool serge, one, *Aspergillus unguis*, caused a pronounced increase in tensile strength and another, *A. niger*, caused a slight though scarcely significant increase; all others caused a definite decrease, with *Fusarium moniliforme* exhibiting stronger action than the others.

TABLE 1

ACTION OF NINE ORGANISMS ON STEAM STERILIZED WOOL SERGE

Organism[1]	Days incubation	Individual breaks, lbs.	Ave.	Visual growth
None; 2 ml. dist. water	10 14	54, 53, 53, 55, 54 57, 50, 56, 56, 55	54 55	0 0
Aspergillus unguis[2] *PQMD 8f*	10 14	70, 67, 67, 70, 64 70, 70, 69, 71, 73	68 71	0 +
Aspergillus niger[2] *PQMD 4j*	10 14	50, 61, 62, 64, — 58, 59, 58, 59, 58	59 58	0 0
Aspergillus ustus[3] *PQMD 4h*	10 14	40, 46, 45, 41, 40 39, 41, 49, 49, 53	42 46	0 +
Aspergillus fumigatus *PQMD 6b*	10 14	38, 38, 38, 48, 42 34, 28, 31, 34, —	41 32	+ +
Fusarium javanicum *PQMD 23d*	10 14	29, 35, 22, 28, 25 29, 28, 31, 34, —	28 31	++ +++
Stachybotrys atra *PQMD 85m*	10 14	18, 25, 26, 45, 28 49, 25, 22, 24, 27	28 29	+ +
Torula convoluta *PQMD 4c*	10 14	25, 19, 19, 19, 19 18, 10, 16, 10, 19	20 15	+ ++
Memnoniella echinata *PQMD 1c*	10 14	15, 19, 15, 17, 15 18, 16, 19, 16, 18	16 17	0 0
Fusarium moniliforme *42 Chem. 70d*	10 14	10, 10, 13, 14, — 12, 14, 15, 12, 11, 15	12 13	+++ ++++

[1] Arranged from top to bottom in order of increasing severity of attack.

[2] Non-cellulolytic.

[3] Of weak cellulolytic ability. All others substantial cellulose destroyers.

2. The two organisms, which in the foregoing test did not bring about any decline in tensile strength of the wool, had been demonstrated previously by repeated tests not to be cellulose destroyers.

Aspergillus ustus, third on the above list, grows well on cotton fabrics with very slow decrease in strength. The six remaining organisms are strong destroyers of cotton fabrics. However, because of the small number of organisms tested on wool. the correlation may be only incidental.

3. Table 1 indicates, in general, little or no decline in strength between the 10th and 14th day of incubation. This is unaccounted for since the two sets of strips appear to have been handled correctly, including breaking by the same individual on the same machine.

TABLE 2

ACTION OF FOUR ORGANISMS ON STEAM-STERILIZED WOOL SERGE

Organism	Days incubation	Lbs. breaking strength; av. of 10 replicates	Visual growth
Control, strips autoclaved, incubated, but not inoculated	7	72	0
	9	64	0
	14	61	0
Aspergillus terreus *PQMD 82j*	7	71	++
	9	62	++
	14	68	+++
Penicillium simplicissimum *USDA 15.1*	7	71	+
	9	65	+
	14	69	++
Pestolotia sp. *Panama 57B5G*	7	69	+
	9	61	++
	14	67	++
Trichoderma viride *PQMD 18e*	7	64	+
	9	57	++
	14	57	+++

Experiment 2. Activity of four miscellaneous fungi on steam-sterilized wool serge (table 2).

The materials and methods were the same as in the previous set of tests. The organisms tested, the periods of incubation, and the results obtained are indicated in table 2.

The following notes were recorded concerning the growth characteristics of the organisms on the wool.

PQMD 82j. Fine white hyphal fuzz developing over whole surface of strip by 7th day and showing a certain amount of scattered pale brown sporulation.

USDA 15.1. Scant white mycelial growth developing slowly with some sporulation at extreme upper end of strip.

Panama 57B5G. White cottony mycelium developing on lower part of exposed portion of strip; no noteworthy increase in growth after 9th day; no sporulation.

PQMD 18e. At 7 days showing a fine fuzzy white mycelial growth more or less evenly distributed over the above-water portion of the strip and a few yellowish fruiting pustules appearing at margins and top; at 9 and 14 days showing considerable white mycelium with yellowish green fruiting hammocks scattered over the cloth.

It is evident from the table and the foregoing paragraphs that although there was enough carbon present, probably as extraneous matter, to enable all of these fungi to make a certain amount of growth, they were nevertheless incapable of attacking the wool directly. It may be noted, incidentally, that all four of these fungi have been shown to break down cotton fabrics under similar test procedures.

Experiment 3. Activity of twelve miscellaneous organisms (including those tested on sterilized wool in experiment 1) on unsterilized wool challie (table 3).

Strips of gray wool challie, raveled to exactly 6 × 1 inches, were wet out in 0.05 per cent Gardinol WA, rinsed in distilled water and placed 1 each in test tubes of 200 × 25 mm. size, each containing 25 ml. of sterile (Greathouse formula A) mineral salts solution. This amount covered a little less than one-half of the strip.

The inoculum was prepared as follows: Each organism to be tested was streaked on a potato dextrose agar slant in a standard 20 mm. test tube and incubated at room temperature for about 3 weeks to obtain good sporulation. To each tube was added approximately 10 ml. of the sterile mineral salts solution.

The spores were loosened and brought into suspension by agitation with a sterile pipette, then the suspension was poured into a

TABLE 3

ACTION OF TWELVE MISCELLANEOUS FUNGI ON UNSTERILIZED WOOL CHALLIE

Organism	Days incubation	Breaking strength, lbs.	Amount of visual growth at end of test
Uninoculated control	6 9 12	34 23 17	0 0 0
Aspergillus fumigatus *PQMD 6b*	6 9 12	32 27 21	0 0 0
Aspergillus niger *PQMD 45d*	6 9 12	36 27 21	0 0 0
Aspergillus unguis *PQMD 8f*	6 9 12	39 14 11	0 0 0
Aspergillus ustus *PQMD 4h*	6 9 12	37 27 22	+ + +
Fusarium javanicus *PQMD 23d*	6 9 12	31 21 11	0 0 0
Fusarium moniliforme *42 Chem. 70d*	6 9 12	37 33 22	0 0 0
Fusarium sp. *PQMD 197*	6 9 12	32 19 9	0 0 0
Gliomastix convoluta *PQMD 4c*	6 9 12	33 16 18	0 0 0
Microsporum gypseum *PQMD 196*	6 9 12	0 0 0	+ ++ ++
Stachybotrys atra *PQMD 85m*	6 9 12	32 25 18	0 0 0
Actinomycete *PQMD 195*	6 9 12	35 32 15	+ + +
Memnoniella echinata *PQMD 1c*	6 9 12	33 27 18	0 0 0

large sterile test tube containing enough sterile mineral salts solution to make the total inoculum up to 35 ml. Two ml. of the suspension were then pipetted evenly over the upper portion of the strip, *i.e.,* the portion which was not submerged in the nutrient solution.

The results are presented in table 3.

In all cases there was a putrid odor and cloudiness in the liquid by the period of the first harvest. In all except *Microsporum gypseum* the results were much the same, *i.e.,* there was steady and pronounced decrease in the tensile strength of the wool strips accompanied by no noteworthy growth of the fungi. There were no noteworthy differences between the sets that were used as controls and those inoculated. *Microsporum gypseum* was in a category by itself with respect to rapidity of action and amount of visual growth produced. It is assumed that in all instances except the *M. gypseum* inoculation, the decline in strength was due to the action of bacterial contaminants. In conclusion, it may be said that under the conditions of this set of tests, wool, which had not been modified by steam heating, proved resistant to degradation by ten common molds and an Actinomycete but was decomposed readily by *Microsporum gypseum.*

Experiment 4. Action of twelve miscellaneous mold fungi on autoclaved as compared with non-autoclaved wool charmeen (table 4).

Strips of gray wool charmeen were raveled to exactly 3 × 1 inches in size. They were dipped in 50 per cent ethyl alcohol, then washed 4 times in distilled water. This was mainly for the purpose of wetting them out. Three variations were introduced into the experiment as will be described further on. Petri dishes of standard 90 mm. size were prepared, each containing a layer of glass beads and 10 ml. of a mineral salts solution. The wool strips were placed two, side-by-side, in each dish. The amount of solution used was sufficient to keep the cloth moist but not submerged. It had the following composition: NH_4NO_3, 3 g.; $MgSO_4 \cdot 7H_2O$, 2.22 g.; KH_2PO_4, 2.59 g.; K_2HPO_4, 2.21 g.; distilled water, 1000 ml.; pH 6.5. With the exception of the controls, 1 ml. of a spore

TABLE 4

ACTION OF TWELVE MISCELLANEOUS MOLDS ON NON-AUTOCLAVED (A) AS COMPARED WITH AUTOCLAVED WOOL CHARMEEN WITH (C) AND WITHOUT (B) YEAST EXTRACT

Organism	Treatment	Tensile strength retained				pH		Observation after 9 days growth
		5 days		9 days		5 days	9 days	
		Lbs.	%*	Lbs.	%*			
Control—not inoculated	A	58		56		6.3	6.45	None
	B	40		33		6.3	6.4	None
	C	39		39		6.4	6.4	None
Alternaria tenuis *PQMD 26a*	A	57	98	58	104	6.4	6.5	Very slight
	B	19.3	49	14	36	7.7	7.7	Moderate; scanty sporulation; purpling of wool
	C	16.1	41	14	36	7.7	7.8	Moderate mycelium and sporulation; deep purpling
Aspergillus fumigatus *PQMD 45h*	A	55	95	53	95	6.5	6.8	Slight growth and sporulation
	B	26	67	20	51	7.9	7.75	Heavy sporulation
	C	23	59	22	56	7.85	7.75	Very heavy sporulation
Chaetomium funiculum *PQMD 33c*	A	54	93	53	95	6.35	6.5	Very slight indication of growth
	B	34	87	31	79	7.0	7.6	Moderate perithecial production
	C	31	79	33	85	7.3	7.5	Heavy perithecial production
Chaetomium globosum *PQMD 79g*	A	54	93	51	91	6.45	6.6	Slight indication of growth
	B	28	72	23	59	7.7	8.0	Moderate growth and perithecial formation
	C	26	67	23	59	7.8	7.9	Heavy mycelium; moderate perithecial formation
Cladosporium herbarum *PQMD 17b*	A	57	98	55	98	6.4	6.4	Slight growth
	B	37	95	35	90	6.5	6.9	Moderate growth with darkening of cloth
	C	38	97	35	90	6.5	6.75	Moderate sporulation

* Note: in calculating the % retention in tensile strength after growth on the autoclaved strips, the figure 39 lbs. was used; for non-autoclaved strips (A) 58 lbs. was used. The figure of 33 lbs. for the 9-day harvest of the control for series B is considered to be in error, possibly due to unobserved bacterial contamination.

TABLE 4—*Continued*

Organism	Treatment	Tensile strength retained				pH		Observation after 9 days growth
		5 days		9 days		5 days	9 days	
		Lbs.	%*	Lbs.	%*			
Fusarium moniliforme *Chem. 70d*	A	56	96	52	93	6.5	6.65	None
	B	11	28	6	15	8.15	8.5	White cottony to slimy growth; cloth brownish
	C	14	36	7	18	8.25	8.5	Heavier growth; cloth brownish
Gliomastix convoluta *PQMD 4c*	A	57	98	57	102	6.4	6.5	Very slight indication of growth
	B	10	26	6	15	7.8	8.0	Moderate growth and sporulation; tan discoloration
	C	13	33	5	13	7.75	7.9	Moderate growth and sporulation; tan discoloration
Memnoniella echinata *PQMD 1c*	A	58	100	57	102	6.4	6.35	Very slight growth
	B	34	87	19	49	7.15	7.5	Very slight growth; brownish discoloration
	C	22	56	14	36	7.4	7.8	Very slight growth; deeper discoloration
Metarrhizium Anisoplae *NRRL 1945*	A	57	98	54	96	6.4	6.4	Very slight growth
	B	29	74	19	49	7.25	7.9	Slight to moderate mycelium; scanty sporulation
	C	28	72	21	54	7.25	7.9	Slight to moderate mycelium; scanty sporulation
Metarrhizium brunneum *NRRL 1944*	A	56	96	53	95	6.35	6.35	Very slight growth
	B	34	87	22	56	6.6	7.5	Moderate growth and sporulation
	C	29	74	19	49	6.8	7.1	Moderate growth and fairly heavy sporulation
Microsporum gypsum *PQMD 196*	A	18.2	31	10	18	7.9	8.1	Moderate white mycelial mat covering cloth
	B	—	—	5	13	8.4	8.3	Heavy white mycelial mat
	C	—	—	7	18	8.4	8.25	Very heavy white mycelial mat
Myrothecium verrucaria *PQMD 185*	A	43.5	75	43	77	7.2	6.9	Slight mycelium and sporulation
	B	19	49	20	51	7.7	8.0	Moderate mycelium; some sporulation
	C	19	49	18	46	7.7	8.1	Moderate mycelium; some sporulation

suspension was distributed by pipette on each strip. The inoculum in each case was prepared from a 19-day-old culture of the organism on potato dextrose agar in a 250 ml. Erlenmeyer flask. Sterile distilled water was used to make the spore suspensions. The dishes were incubated in a high humidity chamber at about 85° F.

For each organism tested 30 strips of wool were used. These were divided into 3 lots of 10 each as follows: (A) strips not autoclaved, (B) strips subjected, after wetting them, to standard autoclaving of 15 minutes at 15 pounds pressure prior to transferring under sterile conditions to the petri dishes, (C) strips autoclaved and yeast extract (0.01 per cent) added to the mineral salts medium on which they were incubated.

Following incubation, the strips were removed from the dishes, conditioned, and broken on the Scott tester as in the previous experiments.

The results are presented in table 4. While all of the fungi tested attacked the autoclaved wool, only two, *Microsporum gypsum* and *Myrothecium verrucaria*, degraded the non-autoclaved wool. Addition of yeast extract was without significant effect except for *Memnoniella echinata* in which case the degradation was accelerated slightly. In all cases where the wool was attacked, the pH increased, the increase in pH being a function of the extent of degradation.

Experiment 5. Action of three fungi on autoclaved wool (table 5).

Results of another experiment carried out the same as experiment 4, but employing only autoclaved wool without yeast extract being added, are shown in table 5. Although *Oöspora lactis,* a strongly proteolytic organism, had no effect, the *Penicillium* and *Syncephalastrum racemosum* degraded the wool slowly.

Experiment 6. Effect of Myrothecium verrucaria on autoclaved and non-autoclaved wool cloth (table 6).

A previous experiment (number 4) had indicated that *Myrothecium verrucaria* could degrade non-autoclaved wool to a slight extent. It was thought that complete degradation of the wool may have been prevented by the high pH of the culture solution after growth had occurred, or from the accumulation of inhibitory waste products. To test this hypothesis a series of cultures was set up employing the same methods as those of experiment 4. Harvests were taken at 3, 6, 10 and 12 days after inoculation (*M. verrucaria, USDA 1334.2*) at which times the culture solution was removed by tipping the Petri dishes and sucking off the liquid with a glass tube connected to an aspirator. Ten ml. of fresh nutrient solution (see experiment 4) were added to each remaining culture. Determinations of pH, using a glass electrode, were made by combining the solutions from the three Petri dishes harvested for each of the two treatments at each harvest. It is evident from the results (table 6) that the limited degradation of wool, resulting from the action of this fungus, is not due to the creation of an unfavorable reaction in the culture solution. The data leave some questions regarding the role of inhibitory waste products since it is possible that the solution held in the cloth strips by capillary action may have contained sufficient amounts to account for the results. It is not believed, however, that the observed limited degradation of the wool is due to this cause. Some other mechanism must obtain. In the processing of wool the fibers are subjected to brief exposure

TABLE 5

ACTION OF THREE FUNGI ON AUTOCLAVED WOOL

Organism	Breaking strength retained				pH		Notes
	5 days		8 days		5 days	8 days	
	lbs.	Ret'n	lbs.	Ret'n			
Oöspora lactis JQMD 433	42	104	38	94	6.45	6.5	No evidence of growth or germination
Penicillium sp. *PQMD 229*	39	96	35	86	6.6	6.6	Some sporulation where chunks of inoculum.
Syncephalastrum racemosum PQMD 57a	39	96	34	84	6.5	6.7	Sparse mycelium; abundant sporangia
Control—not inoculated	40	100	41	100	6.4	6.4	

to heat, alkali and acid. It is quite conceivable that the slight modification of the wool which undoubtedly results from such treatment enables *Myrothecium verrucaria* to grow and that this organism is incapable of attacking undamaged keratin.

Myrothecium verrucaria is commonly isolated from the soil or is found sporulating on plant materials. It is, of course, well known as a strong degrader of cellulose under laboratory conditions. However, there are in the Herbarium of the U. S. Department of Agriculture at Beltsville two specimens which show it inhabiting and sporulating on old cow horn. Both collections are from Louisiana, one taken in 1888 and the other in 1890. One appears under the label *M. roridum* and the other as *M.* sp.

TABLE 6

EFFECT OF *Myrothecium verrucaria* ON AUTOCLAVED AND NON-AUTOCLAVED WOOL CLOTH

Time, days	Autoclaved					Non-autoclaved				
	pH		Tensile strength* retained, lbs.		% retention Inoculated	pH		Tensile strength* retained, lbs.		% retention Inoculated
	Inoc.	Control	Inoc.	Cont.		Inoc.	Control	Inoc.	Cont.	
3	7.4	6.65	30	38	74	6.7	6.55	49	57	86
6	7.6	6.55	23	42	57	6.65	6.5	44	57	77
10	7.35	6.4	16	41	39.5	6.5	6.35	40	57	70
12	6.75	6.35	14.5	—	36	6.25	6.2	39.5	57	69

* Average value of 40 lbs. used for calculating % retention.

Experiment 7. Action of medical fungi and related forms on autoclaved and non-autoclaved wool charmeen (FIG. 1).

Strips of wool cloth (a gray wool charmeen, 70's grade), ¾″ × 4″, were wetted in 50% ethanol, rinsed 4 times in distilled water and placed in 25 × 200 mm. Pyrex test tubes. Half of the tubes were autoclaved (15 lbs. for 15 min.); the remaining tubes were not sterilized. Twenty-five ml. of sterile nutrient solution (see experiment 4) were added aseptically to the tubes. This volume of solution was sufficient to cover about one-half the length of the wool strips. The strips were inoculated in triplicate by drawing a loopful of inoculum upward from the surface of the solution to the top of the strip. Spore suspensions for the inoculum had been prepared by adding sterile distilled water to cultures grown in

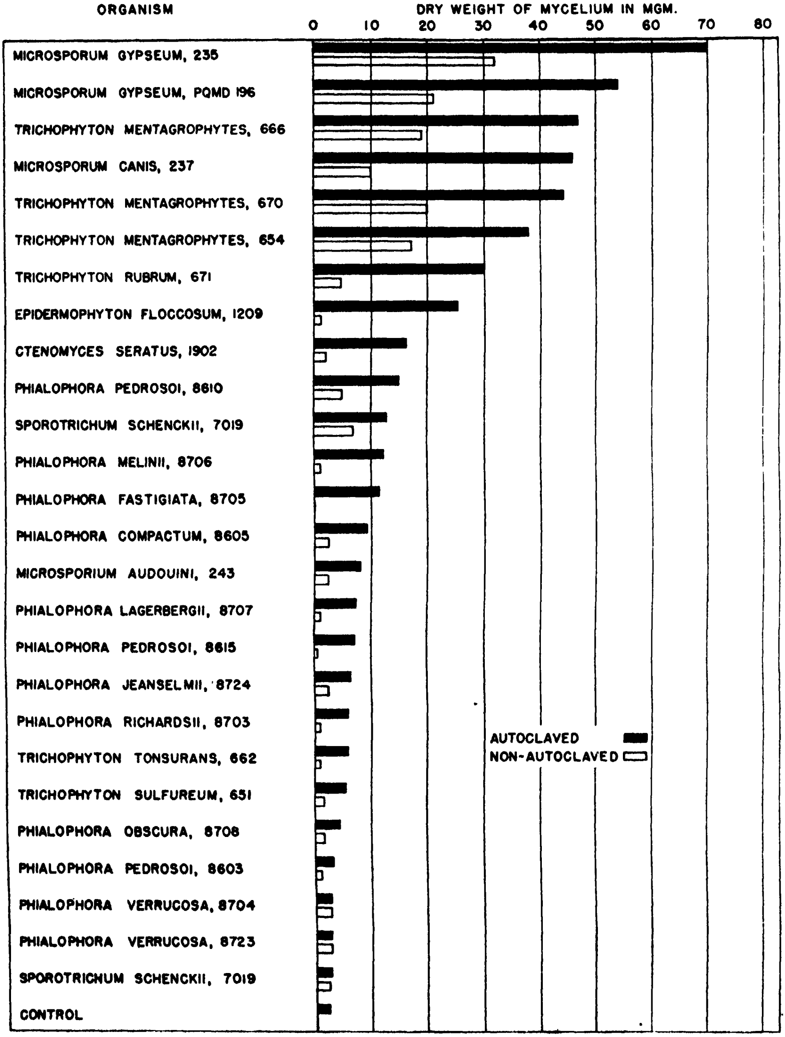


FIG. 1. Growth of dermatophytes and similar or associated fungi on autoclaved and non-autoclaved wool charmeen. The numbers following the names of the organisms, with the exception of the second from the top, are those under which the cultures were received from Dr. Chester W. Emmons of the National Institute of Health.

test tubes on potato dextrose agar for 6 weeks at about 30° C. At the end of the incubation period the Petri dishes and their contents were subjected to autoclaving to kill the organism. The nutrient solution was then decanted off and the wool plus mycelium washed with 0.1 N HCl and distilled water to remove any phosphate precipitate. The contents of the three replicate tubes were then combined, the excess water decanted off and about 50 ml. of boiling 10% NaOH added to dissolve the wool. After cooling, about 60 ml. of distilled water were added and the mycelial residue was filtered off in previously weighed, fritted glass crucibles of medium porosity. The crucibles were then dried overnight at 105° C. and weighed.

The results are presented in figure 1.

The following conclusions may be drawn:

1. Growth of the fungi used varied from luxuriant to little or no growth on both autoclaved and non-autoclaved wool. The dry weight data show similar continuous variation. Thus the differences between the various isolates used are entirely of a quantitative nature, no sharp qualitative differences being observed.
2. Growth on autoclaved wool was greater than that on non-autoclaved wool in all cases where significant growth occurred. No constant relation was found, however, between the two values.
3. With respect to the keratinolytic "activity" of the isolates tested, only certain members of the genera *Microsporum* and *Trichophyton* showed significant ability to degrade wool.

CTENOMYCES-LIKE FUNGI ON WOOLEN FABRICS

Worthy of brief comment at this point is a group of seven isolates having the general morphology of the conidial stage of *Ctenomyces*. All except one of these were obtained from woolen fabrics.

Preliminary work suggests that on the basis of gross colony characteristics correlated with the shape of the conidia the cultures may be segregated into three groups, viz.:

Group A. (FIGS. 2–4)

JQMD 1087. Isolated 14 July 1945 from a woolen overcoat from Guadalcanal.

JQMD 1277. Isolated 21 February 1946 from a woolen overcoat from Guadalcanal (FIG. 2).

PQMD 199. Isolated 5 July 1946 as a contaminant on a strip of gray wool challie that had been inoculated with another organism (FIG. 3).

PQMD 208. Isolated 22 November 1946 as contaminant on strip of gray wool challie that had been inoculated with another organism (FIG. 4).

Group B. (FIG. 5)

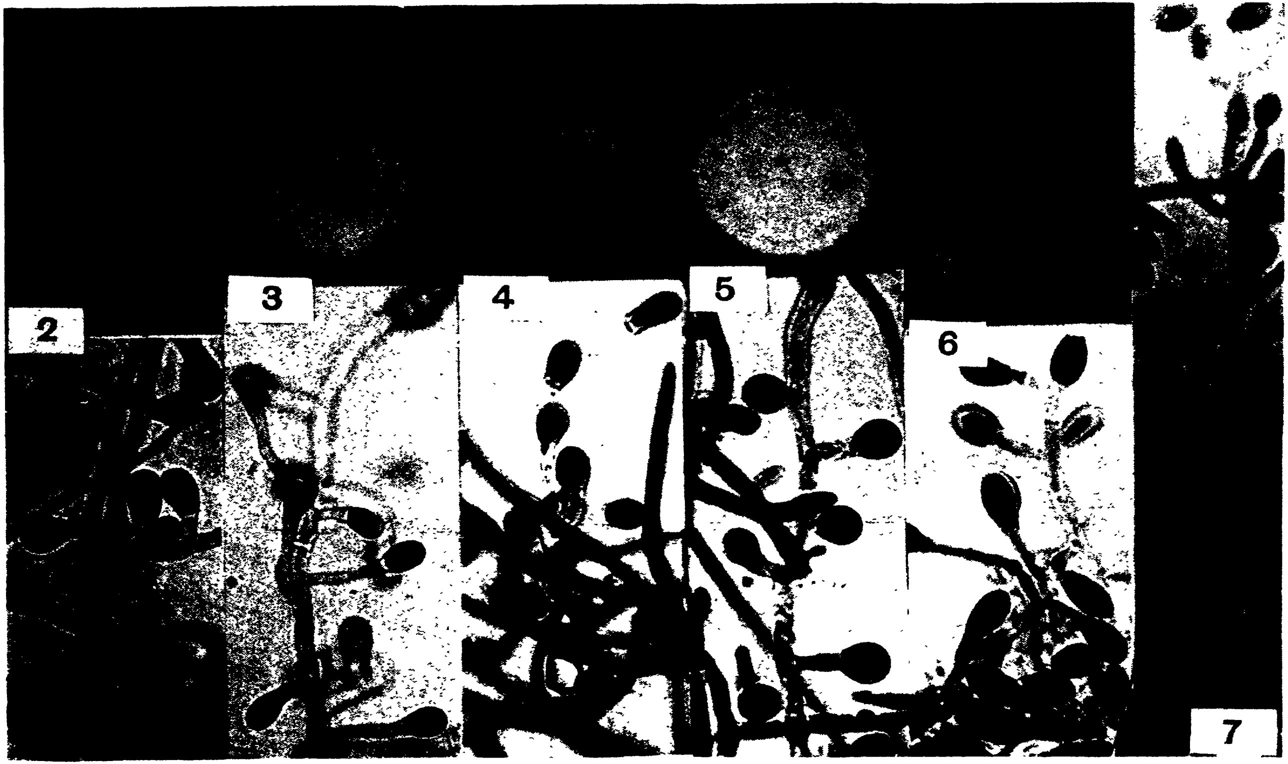
PQMD 287. Isolated from deteriorated wool serge exposed experimentally in a "tropical" room at the British Ordnance Laboratory, Cawnpore, India; culture and sample received at Philadelphia in May 1947.

Group C. (FIGS. 6–7)

JQMD 1070. Isolated 26 June 1945 from a sample of deteriorated tent top from Oro Bay, New Guinea (FIG. 7).

JQMD 1282. Isolated 1 March 1946 from wool serge from Guadalcanal (FIG. 6).

Whether the cultures represent one species or three cannot be determined from the information now available. The photographs in figures 2–6 are of the organisms grown in parallel series for 18 days under room conditions on Bacto Difco potato dextrose agar, 2% dextrose, 35 cc. of the medium in standard 90 mm. Petri dishes. The colonies are white and show about the same growth rate. It is obvious from the figures (2–4) that *JQMD 1277, PQMD 199,* and *PQMD 208* represent one species. The colonies have thin margins and show a tendency for the aerial mycelium of the central areas to sink and become water-soaked. The conidia are obovoid with obtuse, rounded apices. *PQMD 287* (FIG. 5) shows a heavier growth, abrupt margin, no water-soaked appearance in center. The conidia are more broadly obovoid, nearly globose. In *JQMD 1282* (FIG. 6) the colony is striate, and of wholly different texture from the others. The conidia tend to be relatively elongate with the apices pointed. The photomicrograph of *JQMD 1070* (FIG. 7) was taken in a different laboratory from the others and probably



FIGS. 2–7. *Ctenomyces*-like fungi.

under another set of conditions of culture and staining, but it does show clearly the elongate spores corresponding with those of figure 6.

The evidence that these fungi are of significance in wool deterioration is more or less circumstantial. All of the cultures were isolated from wool except one. In the case of those originating at the Jeffersonville Laboratory (*JQMD*) the fabric from which they came was not seen by the writers. In the *PQMD* series, however, the organism was, in each instance, found growing and sporulating on the material. *PQMD 199* appeared on a strip of gray wool challie that had been immersed in Stoddard Solvent for 10 minutes, was then placed on glass beads in contact with a mineral salts solution, and inoculated with another organism that failed to make appreciable growth. In a preliminary test employing autoclaved gray wool challie it caused a more rapid decline in tensile strength than did *Microsporium gypseum, PQMD 196.* Culture *PQMD 208* was obtained under similar conditions to those of *199* but was on several strips of unsterilized and uninoculated experimental control material. Culture *PQMD 287* was received for identification from the British Ordnance Laboratory, Cawnpore, India (their number D-381), accompanied by a sample of olive drab woolen fabric from which it had been isolated and on which it was present in good sporulating condition. It was indicated as having come from a wool serge coat exposed in a "tropical chamber" for test purposes. The letter of communication (from Dr. T. S. Subramanian) referred to it as a wool destroying fungus capable of disintegrating wool fiber completely in the course of a month.

The suggestion that these fungi might have perfect stages in *Ctenomyces* was made by Dr. C. W. Emmons who examined cultures *PQMD 199* and *287.* They have been discussed here because as conidial stages of *Ctenomyces* or some closely related Gymnoascaceous genus they tie in with such genera as *Microsporum* and *Trichophyton* which are also of presumed Gymnoascaceous derivation. It is among such forms as these, as distinct from the great array of common saprophytic molds, that the real degraders of unmodified wool are to be found.

CONCLUSIONS

1. As woolen fabrics are placed on the market—perhaps with the fiber slightly but not seriously modified by prior processing—they are less subject to fungous attack in storage and use than are cotton fabrics.
2. The species of fungi which attack woolen textiles are relatively very few as compared with the range of forms which attack cotton materials.
3. A certain few species of fungi are capable of degrading woolens with a rapidity comparable to the action of the stronger cellulose-destroyers against cotton materials.
4. The fungi that are capable of such activity are commonly found in the conidial stage only, are of similar morphology, belong among, or are closely allied with, the ringworm or skin disease fungi known as dermatophytes, and are probably of Gymnoascaceous derivation.
5. Despite certain reports that are seemingly to the contrary, laboratory tests and field observations by the present writers indicate that the common molds of industrial and military materials do not, as a rule at least, attack wool.
6. Woolen fabrics are modified by standard steam sterilization (autoclaving) in such a way as to render them, in laboratory tests, subject to attack by a wide range of common molds.
7. There is no clear qualitative distinction between those fungi which degrade either or both modified and non-modified wool and those which do not.
8. Degradation is accompanied by an increase in pH, the increase being roughly proportional to the extent of degradation.
9. Woolen fabrics when subjected to the common soil burial tests, where bacterial action is probably of major importance, decay as readily as cotton fabrics subjected to similar conditions.

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DESCRIPTION OF FIGURES

FIGS. 2–7. Ctenomyces-like fungi. 2, *JQMD 1277;* 3, *PQMD 199;* 4, *PQMD 208;* 5, *PQMD 287;* 6, *JQMD 1282;* 7, *JQMD 1070.* All except 7 grown on Bacto Difco potato dextrose agar (2% dextrose) under room conditions for 18 days, the microscopic stages mounted in KOH-phloxine; growth conditions and mounting medium for 7 unknown. Colonies × approx. ½, photomicrographs × 1000. (Colony photos by Photographic Dep't, Phila. Q. M. Depot; photomicrographs 2–6 by Mary Downing, Phila. Q. M. Depot; figure 7 courtesy Biol. Lab., Jeffersonville Q. M. Depot.) For discussion see text.

SOME NOTEWORTHY RUSTS. III *

M. J. THIRUMALACHAR

(WITH 8 FIGURES)

Hapalophragmium mysorense sp. nov.

Pycnia amphigena, subcuticularia, conoidea, applanata, absque ostiolaribus, paraphysibus, evoluta super infectionis maculas hypertrophitas, 2–10 mm. diam. Aecia uredinoidea, amphigena, evoluta, circum pycnia, erumpentia atque pulverulenta, paraphysata; aeciosporae similes urediosporis, 24–28 × 20–24 μ.

Uredia subepidermalia, hypophylla, erumpentia, paraphysata; paraphyses peripherales, tenuiter incurvatae, atque extus projectae, 54–70 × 8–9 μ; urediosporae pedicellatae, evolutae singillatim, luteo-brunneae, ovato-oblongae, 24–28 × 20–24 μ; parietes 1.5 μ crassi, ornati 2–3 germinationis poris equatorialibus, dense echinulati. Telia absque urediis; teliosporae pedicellatae, 3-cellulatae, luteo-brunneae, cellula impari terminali; apice obconico atque protrudenti, ornato germinationis poro in singulis cellulis, 39–54 × 28–40 μ, parietes laeves, sed minutissimis tuberculis ornati prope germinationis porum; pediculis hyalinus deciduus.

In foliis *Derris Benthamii*.

Pycnia amphigenous, subcuticular, conoid, applanate, without conspicuous ostiolar paraphyses, developed on hypertrophied infection spots, 2–10 mm. in diameter. Aecia uredinoid, amphigenous, developed surrounding the pycnia, subepidermal, erumpent and pulverulent, paraphysate; aeciospores resembling the urediospores, 24–28 × 20–24 μ.

Uredia subepidermal, hypophyllous, erumpent, paraphysate; paraphyses peripheral, slightly incurved, and projecting out, 54–70 × 8–9 μ; urediospores pedicellate, developed singly, yellowish brown, ovate-oblong, 24–28 × 20–24 μ, wall up to 1.5 μ thick, with 2 to 3 equatorial germ pores, closely echinulate. Telia developed within uredia, teliospores pedicellate, three-celled, yellowish brown, odd cell terminal, apex obconical and protruding, with an apical germ pore in each cell, measuring 34–54 × 28–40 μ; wall smooth but with warts at the region of the germ pore; pedicel hyaline, deciduous.

Hab. on leaves of *Derris Benthamii* Thw., Kemmangundi, Mysore, 27–4–1949, leg M. J. Thirumalachar (**Type**), type deposited

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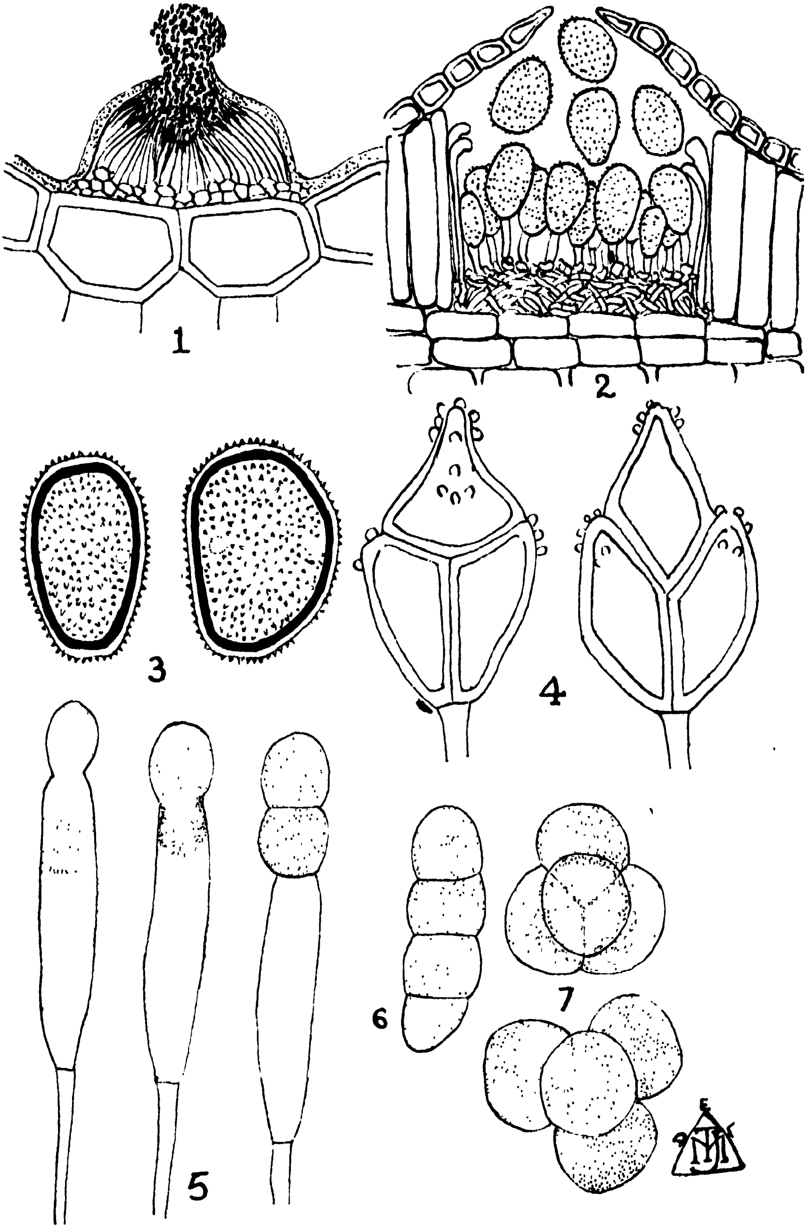
* Part I published in Mycologia **37**: 295–310, 1945; Part II, Mycologia **39**: 231–248, 1947.



FIGS. 1–4. *Hapalophragmium mysorense*; 5–7, *Scopella echinulata.*

in Arthur Herbarium, Purdue University, Lafayette, Indiana, U. S. A.; in Herb. C. M. I. Kew, England, and in Herb. Crypt. Ind. Orient., New Delhi, India (FIGS. 1–4).

There are four other species of *Hapalophragmium* recorded on species of *Derris* with which the present species has to be compared. All the four known species are closely related to each other and show overlapping of characters. Their important characters are summarized below:

Species	Host	Uredio-spore	Telio-spore	Wart in the germ pore of teliospores
H. Derridis Syd.	*D. uliginosa*	27–36 × 19–25 μ	38–58 × 27–38 μ	Not known
H. annamalaiensis Ramakrishnan T.S. & K.	*D. eualata*	24–38 × 19–27 μ	35–58 × 17–34 μ	Absent
H. setulosum Pat.	*D.* sp.	18–20 × 15–18 μ	35–44 × 32–36 μ	Present
H. pulchrum (Racib.) Syd.	*D. elliptica*	20–22 × 18–20 μ	36–42 × 40 μ	Present
H. mysorense Thirum.	*D. Benthamii*	24–28 × 20–24 μ	39–54 × 28–40 μ	Present

It is apparent that the species of *Hapalophragmium* on *Derris* are closely related and show intergrading of characters. *H. mysorense* differs from *H. setulosum* and *H. pulchrum* (which also possess warts near the germ pores) in the size of the uredio- and teliospores.

Subcuticular pycnia and subepidermal uredinoid aecia have been observed in *H. mysorense*. *H. Derridis* Syd. which is the type of the genus *Hapalophragmium*, founded by the Sydows (**1901**), has been pointed out to show close relationship to other species of *Hapalophragmium* on *Derris* including *H. mysorense*. The finding of the pycnial and aecial stage for *H. mysorense* may be taken as representing the condition present in the genus *Hapalophragmium*.

The gall-forming rust *Hapalophragmium ponderosum* has been described by Sydow & Butler (**1912**) from India. Only the telial stage was first described, but later Thirumalachar (**1941**) noted the occurrence of subepidermal pycnia for the rust. This is in contrast to the subcuticular condition just described for *Hapalophragmium* and constitutes a different type of sorus structure. The rust therefore belongs to a separate but closely related genus, and the writer proposes the name *Hapalophragmiopsis* for its accommodation.

Hapalophragmiopsis gen. nov.

Pycnia subepidermalia, conoidea, absque conspicuis paraphysibus ostiolaribus. Aecia atque uredia incognita. Telia subepidermalia, erumpentia, aparaphysata; teliosporae pedicellatae, 3-cellulatae, spora impari terminali ut in Hapalophragmio, luteo-brunneae, singulis teliosporae unico germinationis poro ornatis; pediculus hyalinus et deciduus.

Species typica: *Hapalophragmiopsis ponderosum* (Syd. & Butler) Thirumalachar (FIG. 8, A to D).

Pycnia subepidermal, conoid, without conspicuous ostiolar paraphyses. Aecia and uredia unknown. Telia subepidermal, erumpent, aparaphysate; teliospores pedicellate, 3-celled, odd spore terminal as in *Hapalophragmium*, yellowish brown, with a single germ pore in each cell of the teliospore; pedicel hyaline, deciduous.

Type species: *Hapalophragmiopsis ponderosum* (Syd. & Butler) Thirumalachar on *Acacia leucophloea* Willd.

H. ponderosum causes the production of large gall formations and imparts a striking appearance to *Acacia leucophloea*, a medium-sized tree in India (FIG. 8, A). The galls are produced as a result of infection of the peduncles of the inflorescence and also young fruits. An account of the teliospore germination and pathological histology of the gall was given by Thirumalachar (**1941**). *H. Acaciae* Baccarini is also a gall-forming rust on *Acacia* sp. in Somaliland, Africa. It is reported to resemble *H. ponderosum* closely. This rust may prove to be a species of *Hapalophragmiopsis* when its pycnial stage is discovered.

ON SCOPELLA ECHINULATA (NIESSL) MAINS.

The genus *Scopella* was established by Mains (**1939**) based on *S. echinulata* parasitic on *Bassia latifolia* in India. Subcuticular pycnia, and subepidermal uredia and telia were described, the teliospores being pedicellate, thin-walled and germinating immediately at maturity. The clustering of the urediospores and teliospores on laterally free sporogenous basal cells was noticed by Mains, who

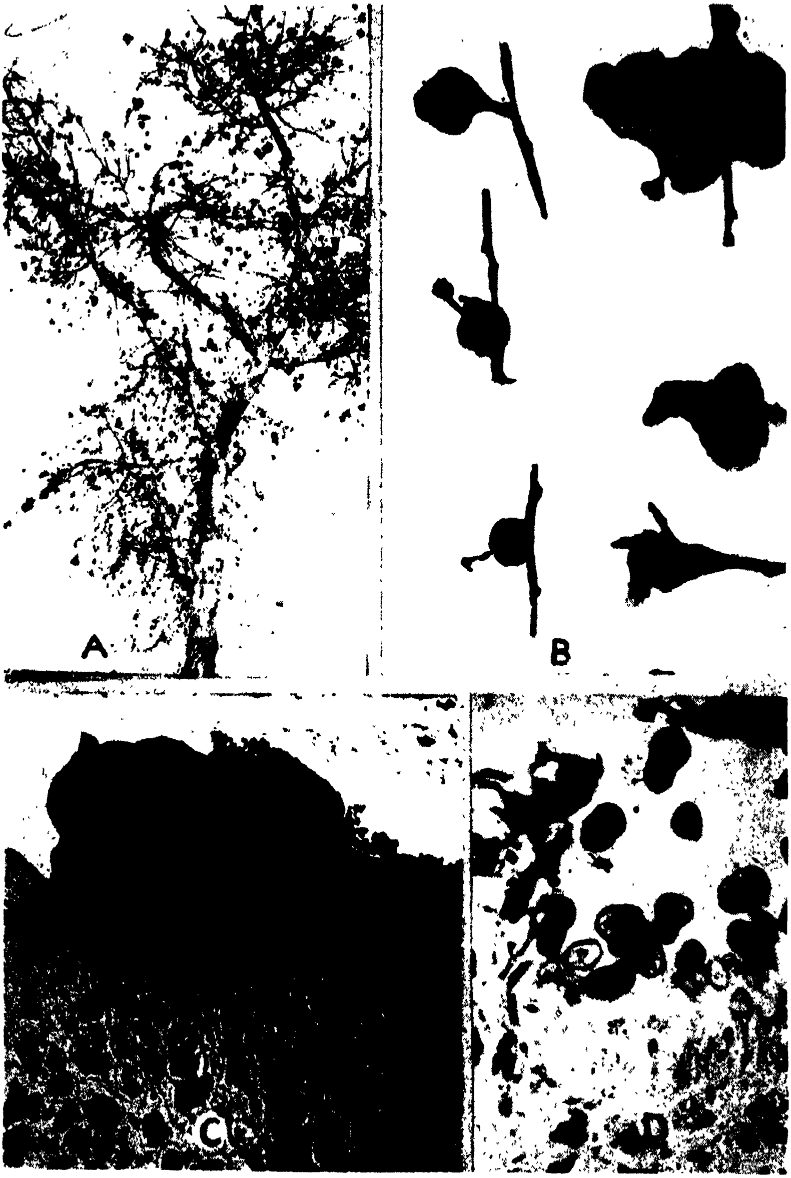


FIG. 8. *Hapalophragmiopsis ponderosum.*

was inclined to adduce much generic importance to such a mode of development. He did not observe the stages of teliospore germination since the spores had collapsed in the herbarium material.

The same rust species has been collected by the writer near Bangalore, Mysore, on *Bassia latifolia* and *B. Bourdilli* which is a new host species. The telia are formed in abundance in the months of December and January and the teliospores germinate immediately intra-sorum.

During the early stages of germination, the tip of the teliospore prolongates into a bud-like structure. There is no promycelium typical of other rusts having four-celled external promycelia. In contrast, the cylindric bud-like structure, into which the contents of the teliospore migrate, may become 1-septate and get separated off (FIG. 5). Further division takes place in this abbreviated promycelium, usually prior to their separation, and results in the formation of four cells. These are arranged in a linear row of four cells (FIG. 6) or, as in several instances, become disposed in a tetrahedral manner due to the plane of divisions (FIG. 7). No sterigmata have been observed and the promycelial cells round off into sporidia.

The tetrahedral disposition of the promycelial cells and their rounding off into sporidia is an interesting feature unknown so far among rust species that have been investigated. In *Coleosporium pulsatillae,* Weir (1912) found that during teliospore germination two intersecting vertical septations took place resulting in a basidium characteristic of the Tremellaceae.

Inoculation experiments have been carried out using the sporidia (scraped from mature sori), on young leaves of *Bassia latifolia* maintained in moist chambers for 48 hours. The inoculation experiments confirmed the autoecious nature of the rust, the pycnia developing on hypertrophied spots after 15 days. Mature pycnia are subcuticular, conoid, applanate, without conspicuous ostiolar paraphyses.

Further studies on such infection spots produced naturally showed the development of uredinoid aecia (primary uredia) accompanying the pycnia. These are subepidermal and without peridium or paraphyses. The aeciospores are identical with the urediospores and borne similarly on clustered pedicels, thus conforming to the fasciculate type of development described by Kuhnholtz-Lordat (**1943**). The uredia (secondary uredia) that follow are hypophyllous and produced on small non-hypertrophied infection spots. The telia are associated with the uredia in early stages and later completely replace them.

The occurrence of uredinoid aecia in the life-cycle of the rust was overlooked by Mains who had access only to herbarium material. The aecia were described as being unknown for the genus. A reexamination of *Scopella gentilis* (Syd.) Mundkur & Thirumalachar and *S. aulicus* (Syd.) Mundkur & Thirumalachar (**1949**), occurring on species of *Mimusops* in India, has shown the occurrence of uredinoid aecia accompanying the pycnia.

UROMYCES LEPTODERMUS Syd. in Ann. Mycol. **4**: 430. 1906. Monogr. Ured. **2**: 334. 1910; Butler & Bisby, Sci. Monogr. No. 1, Counc. Agr. Res. India, p. 82, 1930.

Syn. *Uredo Isachnes* Syd. Ann. Mycol. **4**: 444. 1906.
U. Panici-prostrati Syd. Ann. Mycol. **4**: 444. 1906.
Nigredo leptoderma Arth. North Amer. Fl. **7**: 224. 1912.

Hab. On the leaves of *Brachiaria distachya* Stapf, Bangalore, 6–12–1946, leg. M. J. Thirumalachar; on *Cyrtococcum trigonum,* Yeshwantpur, Bangalore. 8–12–1945, leg. M. J. Thirumalachar.

UREDO CALLICARPAE Petch. in Ann. Roy. Bot. Gard. Peradeniya V, Part IV, p. 251, 1912; Monogr. Ured, IV, p. 420–421, 1924.

Hab. On the leaves of *Callicarpa lanata* Linn., Kemmangundi, Mysore, 26–4–1949, leg. M. J. Thirumalachar.

Uredo Carissae sp. nov.

Uredia hypophylla, minuta, subepidermalia, primo sori evoluti supra epidermidem, et apparenter superficialis, paraphysata; paraphyses cylindricae, incurvatae formantes operculum nido similem 46–60 × 9–10 μ; urediosporae pedicellatae, oblongo-ellipsoideae, materiam aurantiaco-luteum continentes, germinationis poris indistinctis; episporium minute et dense verruculosum; sporae dimetientes 26–34 × 20–24 μ.

Habitat in foliis *Carissae* sp., Kemmangundi, Mysore, 26–4–1949, leg. M. J. Thirumalachar.

Uredia hypophyllous, minute, subepidermal at first, sori formed above the epidermis and thus appearing superficial, paraphysate;

paraphyses cylindric, incurved, forming a nest-like covering 46–60 × 9–10 μ. Urediospores pedicellate, oblong-ellipsoid, with orange-yellow contents; epispore minutely and densely verruculose, with distinct germ pores. Spores measuring 26–34 × 20–24 μ.

On the leaves of *Carissa* sp., Kemmangundi, Mysore, 26–4–1949, leg. M. J. Thirumalachar. Type deposited in Arthur Herbarium, Purdue University, Lafayette, Indiana, U. S. A., Herb. C.M.I., Kew, England, and in Herb. Crypt. Ind. Orient, New Delhi, India.

AECIDIUM PARAMIGNYAE Racib. in Bull. l'Acad. des Sc. de Cracovie, Cl. des Sc. math. et nat. fevrier, p. 277, 1909.

Hab. On leaves of *Paramignya* sp., Balehonnur, Mysore, 4–3–1942, leg. M. J. Thirumalachar, 10–8–1944, leg. K. S. Gopalakrishna.

Pycnia and aecia are present in the material. The aeciospores measure 18–25 × 16–18.5 μ, and the peridial cells are thick-walled, 23–35 × 18–21 μ. *Aecidium Petchii* Sacc. & Trott. is reported from Ceylon on *Paramignya monophylla*, and the Sydows (**1924**) consider that it is probably identical with *A. Paramignyae* Racib.

In conclusion the writer wishes to express his gratitude to Dr. George B. Cummins, Arthur Herbarium, Purdue University, Lafayette, Indiana, for kindly going through the manuscript and for giving the benefit of valuable suggestions, and to Rev. Father Dr. H. Santapau, Professor of Botany, St. Xavier's College, Bombay, for kindly translating the diagnosis of the new genus and species into latin.

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DESCRIPTION OF FIGURES

FIGS. 1–4, *Hapalophragmium mysorense:* FIG. 1. Pycnium, × 400; FIG. 2. Uredinoid aecium, × 400; FIG. 3. Urediospores, × 950; FIG. 4. Teliospores, × 800.

FIGS. 5–7, *Scopella echinulata:* FIG. 5. Germination stages of the teliospore, × 1000; FIG. 6. Showing the linear arrangement of the promycelial cells, × 1000; FIG. 7. Tetrahedral disposition and rounding off of the promycelial cells, × 1000.

FIG. 8, A–D, *Hapalophragmiopsis ponderosum:* A, Infected tree of *Acacia leucophloea*; B, Developmental stages of the rust galls, × nat. size; C, Subepidermal pycnium, × 400; D, Section through telium, × 400.

THE NUTRITION OF MONOSPORIUM APIOSPERMUM

FREDERICK T. WOLF, ROBERT R. BRYDEN AND JOHN A. MACLAREN

(WITH 1 FIGURE)

INTRODUCTION

The disease of man known as maduromycosis, Madura foot, or mycetoma, is due to any one of a number of organisms included among both the actinomycetes and the filamentous fungi. Within the continental United States, the most frequent etiologic agent is *Monosporium apiospermum.*

Boyd and Crutchfield (**1921**) isolated from a case of maduromycosis an ascomycetous fungus which was described by Shear (**1922**) as *Allescheria Boydii.* In a Canadian strain of *M. apiospermum,* Emmons (**1944**) demonstrated the production of perithecia and ascospores similar to those of *A. Boydii,* thus proving that *Monosporium apiospermum* is the conidial stage of *Allescheria Boydii.* The name *M. apiospermum* is commonly retained, however, for those isolates in which the production of the *Allescheria* stage has not been observed.

Little information is available concerning the physiology of this pathogen. Boyd and Crutchfield (**1921**) noted that it could readily be grown in liquid media, and that a number of carbohydrates, including mannitol, galactose, xylose, rhamnose, mannose, lactose, sucrose, maltose and dextrose, were not fermentatively attacked. Benham and Georg (**1948**) have recently investigated the conditions necessary for the production of the perithecial stage in culture, concluding that media rich in organic nitrogen are favorable for formation of conidia but adversely affect perithecial production. These workers cultivated *M. apiospermum* upon a synthetic medium of dextrose agar containing 0.2 per cent asparagine as the only nitrogen source. Thus, the fungus is autotrophic insofar as its requirement for growth factors (vitamins, amino acids, purines and pyrimidines) is concerned.

In connection with observations on various fungi, it was noted that a culture of *Monosporium apiospermum* obtained from Dr. N. F. Conant, Department of Bacteriology, Duke University School of Medicine, grew nicely upon a Czapek's solution of the following composition: $NaNO_3$, 3.0 gm.; KH_2PO_4, 1.0 gm.; KCl, 0.5 gm.; $MgSO_4$, 0.5 gm.; $FeCl_3$, trace; and sucrose, 30 gm. per liter. It was therefore decided to examine certain phases of its nutrition in greater detail.

RESULTS

Carbon Sources: The utilization of a number of carbohydrates, alcohols, amino acids and other organic acids as sources of carbon for *Monosporium apiospermum* was studied using Czapek's solution prepared with the omission of sucrose. To this basal solution were added singly various carbon sources, each in an amount providing 400 mg. of carbon per 100 ml. of medium. Differences in the percentage of carbon among the various substrates were thus taken into account. Cultures were grown in 125 ml. Erlenmeyer flasks containing 50 ml. of solution, adjusted to an initial pH of 5.0.

TABLE 1

GROWTH OF *Monosporium apiospermum* IN CZAPEK'S BASAL SOLUTION (MINUS SUCROSE) CONTAINING VARIOUS SUGARS, ALCOHOLS, AND ORGANIC ACIDS AS CARBON SOURCES

Substrate	Amount of growth	Substrate	Amount of growth
Control (no carbon)	0	Ethanol	++
Xylose	✓++++	Glycerol	++
Arabinose	++	Adonitol	+++
Rhamnose	0	Dulcitol	+++
Glucose	+++	Inositol	0
Levulose	✓++++	Sorbitol	0
Galactose	+++	Mannitol	0
Mannose	+++		
Sorbose	0		
Sucrose	+++	Acetic acid	++
Maltose	++	Malonic acid	+
Lactose	+	Succinic acid	++
Cellobiose	++	Fumaric acid	+
Melibiose	++	Malic acid	+
Trehalose	✓++++	Aconitic acid	+
Raffinose	++	Alpha keto Glutaric acid	+
Melezitose	++	Tartaric acid	++
Dextrin	+++	Citric acid	++
Soluble starch	+++		

Observations as to relative amounts of growth were made after two weeks at room temperature. The results are presented in tables 1 and 2.

It is apparent that *M. apiospermum* is able to utilize a wide variety of compounds as sources of carbon. In general certain of the sugars and amino acids are superior to the alcohols and dicarboxylic acids as carbon sources. Xylose, levulose and trehalose, among the sugars, and alanine, isoleucine and arginine among the amino acids proved to be the most favorable carbon sources. The organism is apparently unable to attack rhamnose, sorbose, inositol, sorbitol, or mannitol, nor can it utilize cystine, methionine, or histidine·HCl as a source of carbon.

TABLE 2

GROWTH OF *Monosporium apiospermum* IN CZAPEK'S BASAL SOLUTION (MINUS SUGAR) CONTAINING VARIOUS AMINO ACIDS AS CARBON SOURCES

Substrate	Amount of growth	Substrate	Amount of growth
Glycine	+	*l*-Arginine·HCl	++++
dl-Alanine	++++	*l*-Lysine·HCl	+
beta-Alanine	++	*l*-Aspartic acid	++
dl-Serine	+++	*l*-Asparagine	++
dl-Threonine	+	*l*-Glutamic acid	+++
dl-Valine	+++	*l*-Tyrosine	+++
l-Leucine	++	*dl*-Phenylalanine	++
dl-Isoleucine	++++	*l*-Tryptophane	+++
l-Cystine	0	*l*-Ornithine	++
dl-Methionine	0	*l*-Proline	+++
l-Cysteine·HCl	+	*l*-Hydroxyproline	+
l-Histidine·HCl	0		

Nitrogen Sources: Experiments to test the suitability of various amino acids as sources of nitrogen for *M. apiospermum* were conducted using a technique analogous to that employed in the experiments with carbon sources. In this case, however, Czapek's medium was modified by omission of $NaNO_3$, and the various amino acids were added in quantities providing 50 mg. of nitrogen per 100 ml. Controls were provided containing $NaNO_3$ or $(NH_4)_2SO_4$. The pH was adjusted to 5.0, and the results obtained after two weeks growth are presented in table 3.

It is apparent that each of the 23 amino acids tested is able to serve as a nitrogen source for *M. apiospermum,* glycine, valine, glutamic acid and tryptophane being most satisfactory for growth of the fungus. Comparison of tables 2 and 3 indicates no correlation between the suitability of a given amino acid as a source of carbon and the suitability of the same compound as a source of nitrogen. *M. apiospermum* is able to utilize both organic (amino) nitrogen and inorganic nitrogen, the latter either as nitrate or ammonium.

Growth in Relation to pH: For studies of the influence of pH on growth of *Monosporium apiospermum*, Czapek's solution was prepared, lacking phosphate, which was supplied by means of McIlvaine's citric acid–Na_2HPO_4 buffers, covering the range of pH 2.2–8.0 at intervals of 0.4–0.6 pH unit. Each flask was prepared to contain 20 ml. of the proper buffer mixture (0.1 M citric acid and 0.2 M Na_2HPO_4) plus the ingredients (minus phosphate) for 50 ml. of Czapek's solution dissolved in 30 ml. The pH of each mixture was checked colorimetrically and the buffer concentration was found to be satisfactory. The cultures were observed after growth for two weeks at room temperature.

TABLE 3

GROWTH OF *Monosporium apiospermum* IN CZAPEK'S BASAL SOLUTION (MINUS $NaNO_3$) CONTAINING VARIOUS AMINO ACIDS, $NaNO_3$ OR $(NH_4)_2SO_4$ AS NITROGEN SOURCES

Substrate	Amount of growth	Substrate	Amount of growth
Glycine	++++	*l*-Lysine·HCl	++
dl-Alanine	+++	*l*-Aspartic acid	+++
beta-Alanine	+	*l*-Asparagine	+++
dl-Serine	++	*l*-Glutamic acid	++++
dl-Threonine	++	*l*-Tyrosine	++
dl-Valine	++++	*dl*-Phenylalanine	++
l-Leucine	+++	*l*-Tryptophane	++++
dl-Isoleucine	++	*l*-Ornithine	++
l-Cystine	+	*l*-Proline	++
dl-Methionine	+++	*l*-Hydroxyproline	+
l-Cysteine·HCl	+	$NaNO_3$	++++
l-Histidine·HCl	+++	$(NH_4)_2SO_4$	+++
l-Arginine·HCl	++		

No growth occurred at pH 3.0 or below. Growth occurred in all instances at pH 3.6 and above, increasing to a maximum at pH 7.0–7.6 and declining somewhat at pH 8.0.

To establish the alkaline limit for growth, additional experiments were performed. Because the buffers customarily used in the pH range 8.0–12.0 contain ingredients such as borate or phthalate which might have effects on fungus growth other than those due primarily to pH, it was decided to employ unbuffered Czapek's solution adjusted to various pH values with concentrated NaOH,

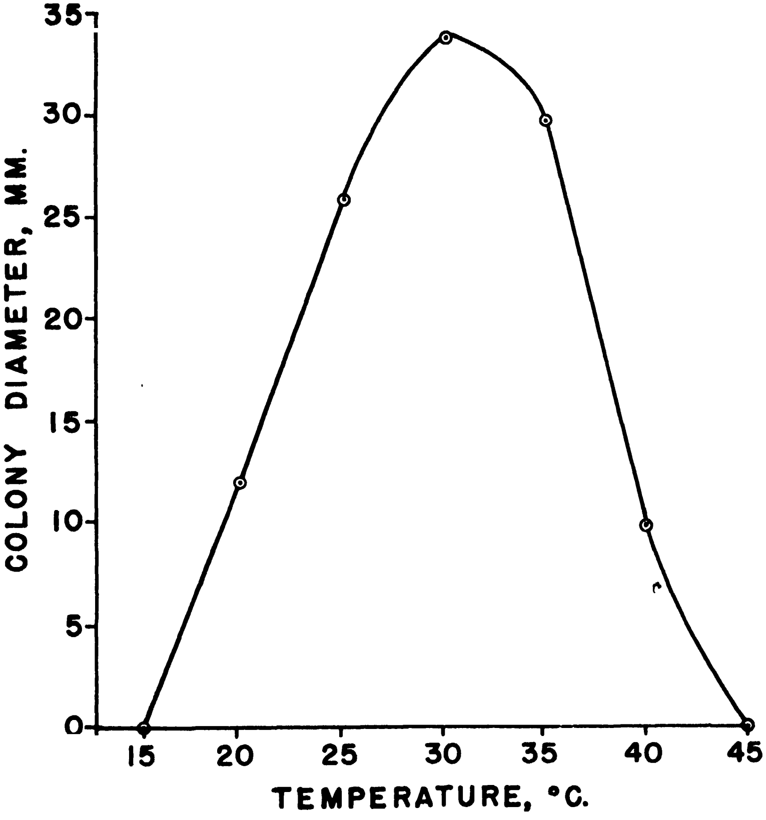

FIG. 1. *Monosporium apiospermum*. Growth in relation to temperature.

and checked by the glass electrode. These experiments indicated that *M. apiospermum* is able to grow up to pH 10.8, but not at pH 11.2 or beyond.

It is evident, therefore, that *M. apiospermum* can tolerate a very wide range of hydrogen ion concentrations, namely pH 3.6–10.8, with optimal growth on Czapek's medium at pH 7.0–7.6, which approximates the pH encountered in its parasitic existence in tissues.

Growth in Relation to Temperature: Studies of the relation of temperature to growth of *M. apiospermum* were performed using plates of Czapek's solution solidified by the addition of 2% agar Plates were inoculated, and held at temperatures of 15°, 20°, 25°, 30°, 35°, 40° and 45° C. for one week, at which time the colony diameters were measured. The results are presented in figure 1.

No growth occurred at 15° or at 45° C. The minimal temperature for growth is therefore between 15 and 20° C. and the maximum between 40 and 45° C. The optimum is close to 30°, somewhat below body temperature.

DISCUSSION

The fact that *M. apiospermum* is autotrophic with respect to its requirements for growth factors is at variance with findings in a number of other fungi pathogenic to man, which have been shown to be deficient for one or more vitamins (Burkholder and Moyer, **1943**; Nickerson and Williams, **1947**). The present findings regarding the carbon nutrition of *Monosporium apiospermum* may be compared with those of Baker and Smith (**1942**) on *Coccidioides immitis,* a pathogenic fungus which has been carefully studied from this standpoint. Of 46 carbon compounds employed by these workers, excellent growth (++++) was obtained with glucose, fructose, mannose, galactose, maltose, cellobiose, trehalose, starch, dextrin, inulin, salicin, amygdalin, ethanol, glycerol, erythritol, mannitol, sorbitol, acetate, succinate, lactate and pyruvate. *C. immitis* does not utilize arabinose, sucrose, lactose, melezitose, inositol, tartrate or citrate, all of which with the exception of inositol are utilized by *M. apiospermum.* Interesting comparisons may also be made with *Aspergillus oryzae,* whose reactions toward 123 different carbon compounds has been studied by Tamiya (**1932**).

The unexpectedly good growth of *M. apiospermum* on the pentose sugar xylose is of particular interest. *M. apiospermum* has not been found in nature apart from clinical infections, known to be acquired from some exogenous source. The present finding indicates that it may exist as a saprophyte in nature upon woody substrata or other plant material.

Little attention has been devoted to amino acids as sources of carbon for fungi. *Fusarium oxysporum* var. *lycopersici* and *Penicillium roquefortii* have recently been examined from this viewpoint by Gottlieb (**1946**), who obtained growth of both fungi with each of 21 amino acids tested, except for cysteine·HCl, cystine, methionine, and tyrosine. *M. apiospermum* is apparently unable to utilize cystine, methionine, or histidine·HCl as a carbon source but grows to a slight extent on cysteine·HCl, and well upon tyrosine. An important factor neglected in the present experiments was the possibility of effects upon growth due to the different isomers of an amino acid, since some of the amino acids used were the naturally occurring isomer and others were racemic mixtures.

An interesting feature of the experiments dealing with nitrogen sources was the finding that *M. apiospermum* is able to utilize each of the 23 amino acids tested as a nitrogen source, while several of these compounds, as noted above, were unavailable to the organism as sources of carbon. The ability to utilize inorganic as well as organic nitrogen would place *M. apiospermum* in Group II of the classification of Robbins (**1937**). Among the pathogenic fungi, the normal form of *Trichophyton mentagrophytes* requires organic nitrogen, although pleomorphic strains can utilize ammonium but not nitrate nitrogen (Robbins and Ma, **1945**). According to Baker and Smith (**1942**), *Coccidioides immitis* is, like *M. apiospermum,* non-exacting in this respect, being able to utilize nitrate, ammonium or organic nitrogen.

The finding that *M. apiospermum* is able to grow throughout a wide range of pH is reminiscent of the results of Leise and James (**1945, 1946**) with *Epidermophyton floccosum* and several species of *Trichophyton*. These organisms were able to grow at pH 10.5, under conditions which suppressed common contaminants.

The finding of an optimum pH for growth of *M. apiospermum* at pH 7.0–7.6 is an apparent exception to the statement that fungi prefer acid substrata. Yet a similar result was obtained by Cross (**1948**) with the yeast-like phase of *Histoplasma capsulatum,* which showed an optimum on several media in the range pH 7.2–7.6, a finding which may prove typical of other systemic fungi.

SUMMARY

Monosporium apiospermum has been grown in Czapek's synthetic nutrient solution. The organism is able to utilize a wide variety of sugars, alcohols, amino acids and organic acids as sources of carbon. Good carbon sources include xylose, glucose, levulose, galactose, mannose, sucrose, trehalose, dextrin, starch, dulcitol, adonitol, alanine, serine, valine, isoleucine, arginine·HCl, glutamic acid, tyrosine, tryptophane and proline. Rhamnose, sorbose, inositol, sorbitol, mannitol, cystine, methionine and histidine·HCl are not available to the organism as sources of carbon.

M. apiospermum is non-exacting in its nitrogen requirement, being able to utilize nitrate, ammonium or amino nitrogen. Growth occurred upon each of 23 amino acids tested as a nitrogen source. Glycine, alanine, valine, leucine, methionine, histidine·HCl, aspartic acid, asparagine, glutamic acid and tryptophane were better nitrogen sources than the other amino acids tested.

This fungus can grow within the pH range 3.6–10.8, with an optimum pH of 7.0–7.6.

Growth of *M. apiospermum* occurs between a lower temperature limit at 15–20° C. and an upper limit at 40–45° C., with an optimum at approximately 30° C.

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A STUDY OF AN ISOLATE OF BREVILEGNIA FROM NEW CALEDONIA

T. W. Johnson, Jr.[1]

(WITH 1 FIGURE)

During the progress of a study of fungi isolated from soil samples sent by Professor J. T. Buchholz from New Caledonia to the Mycological Laboratories, Department of Botany, University of Illinois, a species of the genus *Brevilegnia* was obtained which appeared to be different from any of the hitherto described species in the genus. An investigation by Salvin (4) of an isolate of *Brevilegnia* obtained from Cuba led him to conclude that specific and varietal characteristics in the genus were so variable that only two species appeared valid, and that these should be transferred as a sub-genus to the genus *Thraustotheca*. It seemed obvious, therefore, that a designation of the New Caledonia isolate as a new species had to be done with a consideration of the possibility of variation.

The conclusions presented by Salvin were arrived at by the study of one isolate only, and the observations were not supported by a comparative study of isolates whose characteristics under standardized cultural conditions were those of previously described species. Since such comparisons were lacking, it is possible that only his isolate could be characterized by such extreme variability, and that more stable forms existed which should be recognized as valid species. Therefore, a study was initiated (a) to determine if the New Caledonia isolate possessed variability comparable to Salvin's isolate, and (b) on the basis of results obtained, to determine if it could be regarded as representing a variant of one of the accepted species of *Brevilegnia* or as a new species.

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[1] The author wishes to express his sincerest gratitude to Professor Leland Shanor, for his guidance and helpful suggestions in the course of the study, and in the preparation of this paper. The author's present address is, Department of Botany, University of Michigan, Ann Arbor, Michigan.

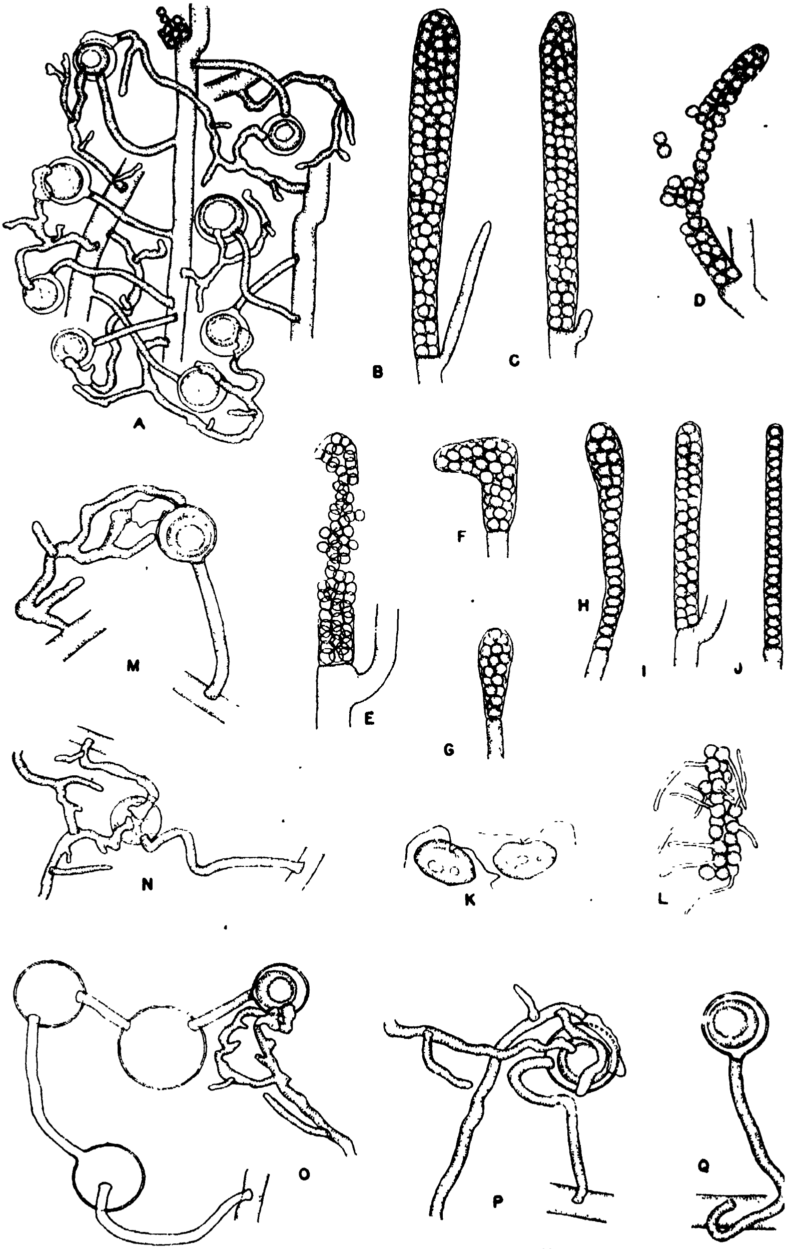

FIG. 1. *Brevilegnia longicaulis.*

Each isolate employed in this study was derived from a single, germinated zoospore, and was maintained in culture growing on boiled, split hempseed in 30 cc. of sterile, charcoal-filtered, distilled water at a temperature of 22 ± .3° C. Assignment of specific names to all isolates of other species of *Brevilegnia* at hand was done on the basis of the characteristics of those isolates when grown in such cultures, since the original descriptions of species assigned to the genus were based either wholly or in a large part upon isolates grown in this manner. The details of culture methods employed for the study of possible variations under differing environmental conditions will be described under the section dealing with experimental studies. This paper presents the results of these studies, and in view of these results, the New Caledonia isolate is believed to represent an undescribed species.

Brevilegnia longicaulis sp. nov.[2] Mycelium spissum, breve, quod abundantes in *Cannabis sativae* seminibus gignitus. Hyphis plerumque non ultra 5.0 mm. longis, crassis, et ad basim ramosis; mediis hyphis plerumque 24.7 μ diam. Sporangiis copiosis, clavatis in longis cylindricalibus, 167–301 μ longis, 22.1–26.8 μ diam.; terminalibus, cymosis. Sporis plus v. minus angularibus, plerumque 9.1 μ diam., quae aut in situ germinant aut sporas motiles secundarias proferunt. Oogoniis copiosis, singulis et ad ramuli partem extremans affixis; 24.8–29.0 μ diam.; sphaericis; tunicas leves, tenues, hyalinas, et nulla papilla alterave parre prominente deformatas habentibus. Oosporis 22.5–26.0 μ diam., sphaericis, quae singulae singulis in oogoniis ita gignuntur ut oogonia non impleant et paulo ab oogonii centro absint. Episporio levi, crasso. Antheridiis diclinis, longis, haud multum ramosis, quae plerumque singula, nonnumquam bina, terna, vel quaterna in oogoniis gignuntur sed interdum omnino desunt. Nullis gemmis.

Hab.: ad terram humosam, Köe Dumbra, New Caledonia, September 23, 1947.

Mycelium depauperate, forming a dense growth on hempseed. Hyphae usually not more than 5.0 mm. long, averaging 24.7 μ in diameter near the center; delicate and branching. Sporangia abundant, terminal, clavate to long-cylindrical, 93–375 μ long by 21.8–53.6 μ in diameter, averaging 167–301 × 22.1–26.8 μ; generally 3–5 spores wide, not branching, not producing spores in a single row either in part or in the whole; secondary sporangia arising by cymose branching. Encysted spores spherical to angular, 6.2–11.0 μ in diameter, averaging 9.1 μ; encysting within the

[2] Latin diagnosis prepared with the assistance of Professor R. P. Oliver, Department of Classics, University of Illinois.

sporangium, and escaping upon disintegration of the sporangial wall, then leaving the cysts as motile spores; only rarely escaping from the cysts while these are still united in a partly disintegrated sporangium, and then leaving a partial false-net cluster of cysts; occasionally germinating *in situ*. Oogonia abundant, spherical, 22.5–33.3 μ in diameter, generally 24.8–29.0 μ; arising singly on long, slender, lateral stalks; wall smooth, thin, hyaline, unpitted; immature oogonia frequently proliferate until an antheridium becomes attached. Oospores 1 to each oogonium, spherical, and not filling the oogonium; eccentric, with a large oil drop entirely surrounded by protoplasm; 20.1–27.5 μ in diameter, generally 22.5–26.0 μ. Antheridial branches diclinous, long, irregular, and branched; never observed androgynous, and seldom not developing; one, rarely two to four antheridia to the oogonium; antheridial cell broadly attached to the oogonium; antheridial tubes not observed. Gemmae lacking.

From soil collected from the edge of a brook in a ravine, approximately 5 miles east of Köe Dumbra, New Caledonia, September 23, 1947, by Professor J. T. Buchholz.

Slides of preserved material from the type culture are being deposited in the herbaria of the University of Illinois, and the University of Michigan.

For purposes of distinguishing *Brevilegnia longicaulis* from other members of the genus which have been described, we have used living cultures, collected in Illinois by Dr. E. S. Beneke, of *Brevilegnia unisperma*, *B. unisperma* var. *delica*, *B. unisperma* var. *montana*, *B. unisperma* var. *littoralis*, and *B. diclina*, and have compared our isolate with the original descriptions of *B. bispora*, *B. linearis*, *B. subclavata*, and *B. megasperma* (**1**, **2**, **3**). In view of the possible variations which may exist in sporangial characters, prime consideration was given to fundamental differences in sexual reproductive structures. Salvin has indicated that even in his variable isolate, he considered such features as the type of antheridium and general morphology of the oogonium to be genotypically determined. These structures, then, it would seem, are more constant than sporangial characteristics for determining specific differences. With this consideration, and on the basis of the comparisons made, *Brevilegnia longicaulis* differs from any of the previously described species and varieties in the genus.

EXPERIMENTAL STUDIES

Six control cultures, each consisting of one-half of an infected hempseed, were employed for each phase of the experimental study. These cultures were propagated as single spore isolates in 30 cc. of sterile, charcoal-filtered, distilled water at a temperature of 22°± .3° C. Comparisons of the experimental colonies and the control colonies were made when both were of approximately the same age. Although only one culture on hempseed was used for each environmental condition, all experiments were repeated several times. Unless otherwise specified, the results herein reported are based on the average of 200 observations or measurements in each culture observed. Growth under any of the conditions not discussed below was similar to growth in the controls.

Effect of environment on sporangial morphology: As suggested by the procedure outlined by Salvin in his investigation of *Brevilegnia* isolate *C–2*, *Brevilegnia longicaulis* was grown in varying amounts of sterile, charcoal-filtered, distilled water, with and without aeration, at temperatures ranging from 10 to 35° C. (at intervals of 5° C.), and in water containing the staling products from several cultures of the isolate. The volumes of water used were 1, 5, 10, 25, 50, 100, 300, 500, 1000, 1500, and 2000 cc. The staling water employed was obtained by filtering, through a porcelain filter, water in which cultures of *B. longicaulis* had previously been grown. Twenty colonies, each one week old, were placed in 50 cc. of sterile, charcoal-filtered, distilled water, and allowed to develop for a period of 2 weeks. This procedure was repeated several times until sufficient staling water had been obtained. Cultures were grown in 25, 50, 100, and 200 cc. of this water, without aeration, at a temperature of 25° C. The effects of temperatures of 2, 5, and 7° C. on sporangia of cultures grown in volumes of water of 25, 50, 100, 200, 300, 500, and 1000 cc. were also studied. In all cases, observations were made on sporangial shape and size.

In the case of cultures grown in 1 and 5 cc. of water, without aeration, at temperatures of 25, 30, and 35° C., sporangia were somewhat shorter than those of the control cultures. The range of the measurements of these sporangia was 67.5–181.5 × 22.5–23.1 μ, as compared with the range of 171.5–297.0 × 22.1–27.6 μ for the controls, and they were typically clavate in shape. In quantities of water from 10 to 500 cc., without aeration, at temperatures of 25, 30, and 35° C., sporangia showed some variation, the most noticeable being a slightly smaller diameter, with a tendency, in 115 of the sporangia measured, to be mostly 2 spores wide (FIG. *I*). Sporangia in these cultures measured 187.5–202.0 × 19.1–21.5 μ, and were usually long-cylindrical in shape. In volumes of water above 500 cc., without aeration, sporangia were similar to those in the controls, except for a few sporangia in the cultures grown at 25° C. in 1000 cc. of water, these being abnormal in that the tips were bent as indicated in figure *F*. As far as could be observed, temperature in the range employed did not appear to have any pronounced effect on sporangial morphology.

In the experiments involving aeration in addition to the volumes of water and temperatures as listed above, cultures grown in 25, 50, and 100 cc. of water at a temperature of 25° C. produced sporangia which, in 2.2 per cent of the 300 sporangia observed, were longer and more cylindrical than those in non-aerated cultures. This degree of variation appears negligible, however, when compared with the variation of 60.8 per cent which Salvin obtained with isolate *C–2,* when grown under comparable conditions. In the temperature range between 10 and 25° C., in aerated water, regardless of amount, sporangia were shorter, but remained essentially clavate in shape. The significance of this variation at lower temperatures in aerated cultures has not been determined. The variation is interesting, however, in view of the lack of such variation when the water was not aerated.

Cultures of *Brevilegnia longicaulis* were also grown in 4000 cc. of water, with aeration, at a temperature of 25° C. Examination of 255 sporangia from these cultures indicated that the only variation induced in these structures was a decrease in their diameter. The average diameter of these sporangia was 17.3 μ, but in respect to length and shape, the sporangia were similar to those in the controls. Repetitions of the tests employing 4000 cc. of aerated water revealed the following variations: (a) the production of sporangia which were 1-spore wide in the basal half, and 2–3 spores wide in the apical half, in about 16 per cent of 205 sporangia observed (FIG. *H*), and (b) the production of sporangia which were 1-spore wide throughout their entire length (FIG. *J*). This extreme narrowing, not observed in any other cultures, occurred in approximately 12 per cent of 200 sporangia observed.

The study on *Brevilegnia C–2* showed that in that isolate, at least, temperatures below 10° C. inhibited the full development of the sporangia, and any visible development was limited to a simple swelling of the hyphal tips. Cultures of *B. longicaulis* held at 2° C. failed to develop any sporangia, and after two weeks in this environment, examination revealed that no swelling of the hyphal tips had occurred. Attempts to obtain growth from these cultures by hyphal tip transfers onto MP-5 Agar were unsuccessful, and it is presumed that the mycelium was killed by the low temperature. At 5 and 7° C., however, the hyphal tips showed some swelling in the form of elongate enlargements suggesting the shape of the typical clavate sporangia. Development beyond this stage was not observed.

Staling water was utilized in an attempt to induce variations in sporangial shape. Salvin reported that sporangia of *Brevilegnia C–2,* developed on cultures growing in concentrated staling water, without aeration, for 10–14 days, were almost "exclusively short and clavate," with only a "few having the spores arranged in a linear series" (4, p. 42). The number of spores in the sporangia in his cultures ranged from 4–16. Of the 160 sporangia of *B. longicaulis* observed in cultures grown in 50 cc. of unaerated staling water at a temperature of 25° C., 74.2 per cent were typical in size, whereas the remainder were shortened, ranging from 31.0–87.5 μ in length (FIG. *G*). The number of spores in these sporangia was large in comparison with the number of spores reported for *Brevilegnia C–2.*

Effect of the environment on the production of gemmae: Cultures of *Brevilegnia longicaulis* were grown for 12 days in 25 cc. of staling water (obtained by the same procedure outlined for the study of sporangial variations) at a temperature of 25° C., but in no cultures were gemmae produced. Similarly, older cultures of *B. longicaulis,* growing in the water in which they were originally placed, did not form gemmae. Salvin, however, was consistently able to induce the formation of gemmae by the use of concentrated staling water, even though they were not formed, in his isolate;

on cultures in "pure water" (4, p. 44). The results obtained with our isolate would tend to indicate that, in this species at least, the formation of gemmae may be genetically controlled and not influenced by an environmental factor.

Effect of the environment on antheridial formation: In the report on the investigation of *Brevilegnia C–2*, the amount of available oxygen was found to be the factor contributing most to variations in antheridia. Again utilizing Salvin's procedure, colonies of *B. longicaulis* were suspended at various depths of water from the surface to 12 inches, at intervals of 1 inch, and temperature was maintained at 25 ± .5° C. Standard glass cylinders were used which measured 35 mm. inside diameter. After 10 days the cultures were examined for variations in the antheridia, both in percentage formed and for origin. No attempt was made to determine, quantitatively, the actual degree of aerobiosis in the culture tubes.

Of the oogonia of *B. longicaulis* formed at the 10-inch level, 61.3 per cent had antheridia attached, 38.7 per cent had no antheridia. Of the oogonia produced in the cultures growing at the 5-inch level, 63.9 per cent had antheridia attached, the remainder lacked them, and of the oogonia produced at surface level, 69.6 per cent had antheridia. For comparison, control cultures produced oogonia 72.0 per cent of which had antheridia, whereas the remaining 28 per cent of the oogonia lacked these structures. Briefly, the results of Salvin showed that with increasing depth at which the mycelium of *Brevilegnia C–2* developed, fewer antheridia were formed. A culture of *Brevilegnia C–2* growing at the surface of a 10-inch column of water had 82 per cent of the oogonia with antheridia attached, but a culture growing at the 10-inch depth of the column had only about 4 per cent of the oogonia with antheridia.

The effect of the degree of aerobiosis on the origin of the antheridial branches was determined by observations of these same cultures. The percentages of antheridia developing diclinously, androgynously, or not at all, were very similar in the 10-inch, 5-inch, and surface levels to the percentages developing in the standardized control cultures: diclinous, 71.1 per cent; androgynous, 0.0 per cent, and not developing, 28.9 per cent. These results agree with those obtained by Salvin in that he reported that characters such as type of antheridium (diclinous or androgynous) were not proven to vary with the environment, but rather, were apparently determined genetically.

Although not included in Salvin's study of *Brevilegnia C–2,* the characteristics of *B. longicaulis* when grown in more natural environments were investigated. Fifteen separate single spore isolates of *B. longicaulis* were grown in 15 samples of water and debris. These samples were selected from such diverse locations as cold, clear, running water; cold, stagnant water, and warm stagnant water. After two weeks in these "natural habitats," the cultures were observed, particularly for changes in the distinguishing characteristics. With the exception of minor variations in the morphology of sporangia (these were usually longer, and a greater number of them had the encysted spores germinating *in situ*), the features of the fungus were not obviously altered.

DISCUSSION

In the recent paper by Salvin (4), reporting the effects of various environmental factors on the characteristic morphological structures that distinguish the genus *Brevilegnia,* as represented by his isolate, *Brevilegnia C–2,* the variation in sporangial size and shape, formation of gemmae, and the presence or absence of antheridia, were evaluated. Particular attention was paid to the duplication of the various sporangial characteristics that have distinguished most of the previously described species belonging to the genus, and Salvin concluded from his results that only two of all the species and varieties that had been reported should be retained as specific entities. *Brevilegnia bispora* was retained, but all of the others were regarded as merely representing variations of *B. unisperma* that could be induced by varying the environment in which the cultures were grown. Furthermore, Salvin proposed the reduction of *Brevilegnia* to a sub-genus of *Thraustotheca,* designating the species in the sub-genus as *Thraustotheca bispora* and *T. unisperma.*

On the basis of the results of a study of an isolate of *Brevilegnia,* herein reported as *Brevilegnia longicaulis,* we are not prone to accept all of the conclusions reached by Salvin. In the first place, our isolate, *B. longicaulis,* does not exhibit the wide variability in sporangial and other characters which Salvin reported for *Brevi-*

legnia C–2. Within reasonable limits, our isolate seems to be a very stable organism. Should the same be true for the other species of *Brevilegnia,* there would be no justifiable reason for grouping these under the specific name *Brevilegnia unisperma.* Before Salvin's conclusion in this regard is accepted, it would be highly desirable to study the other species of the genus to determine if they follow the pattern of variability exhibited by his one isolate. Indeed, *Brevilegnia C–2* may only exhibit a degree of variability not infrequently encountered in selected isolates representing other species of fungi.

Secondly, we see nothing gained by reducing *Brevilegnia* to subgeneric rank under these circumstances. When the genus was established by Coker and Couch (1), the similarity of certain of the sporangial characters in *Brevilegnia* and *Thraustotheca* was recognized. Other characteristics, however, such as the single oospore, the depauperate nature of the mycelial development, and the behavior of the spores in *Brevilegnia,* were regarded as the basic characters separating this genus from the genus *Thraustotheca.* Salvin's results have in no way altered this original hypothesis. Therefore, we prefer to retain *Brevilegnia* as a genus.

On the basis of our study of *Brevilegnia longicaulis,* we are able to concur fully with Salvin that the type of antheridium, the general morphology of the oogonium, size of oospores, and the method of sporangial proliferation are stable features, and it is primarily on the basis of these that the isolate we have studied has been distinguished from the other species of the genus.

SUMMARY

A new species of *Brevilegnia* is described as *Brevilegnia longicaulis.* The fungus was recovered from a soil sample obtained along a ravine near Köe Dumbra, New Caledonia. It is characterized by clavate to long-cylindrical sporangia renewed by cymose branching, motile zoospores, diclinous antheridia, proliferating oogonia, and oospores which are consistently larger than those in species and varieties of *Brevilegnia* previously described, with the exception of *Brevilegnia megasperma.*

The fungus was subjected to extensive experimentation in an attempt to induce specific variations in the morphology of the spo-

rangia, the origin of the antheridia, and to induce the formation of gemmae. Variations obtained were slight, and not sufficiently pronounced to conclude that this species was merely a variant of any other species.

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DESCRIPTION OF FIGURES

Brevilegnia longicaulis. FIG. *A.* Portion of mycelium growing on hempseed, showing long oogonial stalks, and diclinous, irregular and branched antheridia. × 840. *B.* Mature sporangium of the characteristic clavate type. × 840. *C.* Mature sporangium of the typical long-cylindrical type. × 840. *D.* Mature sporangium showing disintegration of wall, and liberation of encysted spores. × 840. *E.* Sporangium in which some of the encysted spores have been liberated normally, the remainder having emerged from their cysts, leaving a partial false-net sporangium. × 840. *F.* A bent sporangium formed in a culture grown at 25° C. in 1000 cc. of unaerated water. × 840. *G.* A short-clavate sporangium formed in a culture grown at 25° C. in 50 cc. of unaerated staling water. × 840. *H.* A modified sporangium with spores in a single row in the basal half, observed in cultures grown at 25° C. in 4000 cc. of aerated water. × 840. *I.* A narrow-cylindrical sporangium 2 spores wide, formed in a culture grown at 30° C. in 100 cc. of unaerated water. × 840. *J.* A modified sporangium 1 spore wide, formed in a culture grown at 25° C. in 4000 cc. of aerated water. × 840. *K.* Motile stage of zoospores after emergence from cyst. × 1980. *L.* A cluster of encysted zoospores, germinating *in situ.* × 840. *M.* Typical mature oogonium showing diclinous antheridial branches, attachment of antheridia, and mature oospore. × 1180. *N.* Immature oogonium with attached diclinous antheridial branch. × 840. *O.* Proliferation of an oogonium, showing the ultimate, matur[illegible] with a diclinous antheridium. × 1180. *P.* Mature oogonium with 2 [illegible]ed antheridial branches. × 1180. *Q.* Mature oogonium which has develop[illegible]arthenogenetically. × 1180. All figures made with aid of a camera lucida.

THE ACTIVITY IN VITRO OF CYCLOHEXIMIDE (ACTI-DIONE[1]) AGAINST FUNGI PATHOGENIC TO PLANTS

ALMA J. WHIFFEN

(WITH 1 FIGURE)

The demonstration by Dr. Irma Felber and Dr. Charles Hamner (1948) that powdery mildew (*Erysiphe polygoni* DC.) on bean plants in the greenhouse could be controlled by the antibiotic cycloheximide in concentrations from five to ten parts per million has aroused interest in the effectiveness of cycloheximide against other plant pathogens. Earlier, cycloheximide had been shown to be active against a number of yeasts but only slightly active against the filamentous fungi that cause disease in animals (Whiffen, 1948). In this paper the spectrum of antibiotic activity of cycloheximide *in vitro* is extended to cover twenty-nine species of fungi pathogenic to plants.

MATERIALS AND METHODS

With the exception of cultures obtained from culture collections, as noted, all species tested were freshly isolated from diseased plants. Isolation of the pathogenic fungi was greatly aided by the addition of streptomycin, in concentrations from 100 to 300 micrograms per milliliter, to plates of Blakeslee's medium (Difco malt extract 20 grams, Bacto-peptone 1 gram, dextrose 20 grams, agar 20 grams, distilled water 1000 milliliters). These concentrations of streptomycin suppressed or inhibited the multiplication of the majority of contaminating bacteria without having any inhibitory effect upon the growth of the fungi.

Eleven species were obtained for us by Dr. Ralph Lewis from individuals and culture collections as follows: *Sclerotinia fructicola*,

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[1] Cycloheximide has been assigned as the generic name for [illegible]mpound β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarim[illegible] for which The Upjohn Company adopted "Acti-dione" as its trademar[illegible]

Alternaria solani, and *Alternaria oleracea,* S.E.A. McCallan, Boyce Thompson Institute; *Sclerotium rolfsii,* W. D. McClellan, Bureau of Plant Industry, Beltsville; *Pythium debaryanum* and *Diplodia zeae,* Benjamin Koehler, University of Illinois; *Macrosporium sarcinaeforme,* J. G. Horsfall, Connecticut Agricultural Experiment Station; *Physalospora tucumanensis* Light Strain and Dark Strain, C. W. Edgerton, Louisiana State University; *Venturia inaequalis* 365–4, G. W. Keitt, University of Wisconsin; *Diaporthe citri,* L. J. Klotz, Citrus Experiment Station of the University of California, Riverside, California.

Crystalline cycloheximide was diluted in plates of D.P.Y. agar medium to give concentrations of 100.0, 40.0, 20.0, 10.0, 5.0, 2.5, 1.25 micrograms per milliliter and 1.0, 0.5, 0.25, 0.125, 0.062 microgram per milliliter. The composition of D.P.Y. medium is as follows: dextrose 10 grams, Bacto-peptone 5 grams, Difco yeast extract 1 gram, agar 20 grams, distilled water 1000 milliliters; pH 6.8. Spore suspensions of the fungal pathogens were prepared from ten-day-old agar slant cultures on Blakeslee's medium and streaked on the surface of plates containing cycloheximide. Those fungi that did not sporulate on agar, *e.g., Pythium debaryanum, Rhizoctonia* sp., and *Sclerotium rolfsii,* were grown on agar plates and a disc of mycelium was cut from the margin of the colony for inoculum. The concentration at which there was complete inhibition of growth was recorded after 48 hours of incubation at 24° C. and again after 72 hours. The tests were replicated four times with four different lots of spores.

To determine the effect upon the rate of mycelial growth, cycloheximide was diluted in D.P.Y. agar to cover a range of concentration from 0.0075 to 4.0 micrograms per milliliter, using a dosage ratio of 2.0. Duplicate plates were inoculated in the center with an agar disc, four millimeters in diameter, cut from the edge of a forty-eight-hour-old culture of *Pythium debaryanum.* The plates were incubated at 30° C. and at intervals of 12 to 24 hours, the margin of the colony was marked along previously ruled radii. The rate of growth in terms of millimeters per hour was determined for each concentration of cycloheximide and for the control lacking cycloheximide and from these data the percentage of retardation of mycelial growth from that of the control was calculated. The experiment was replicated five times. This method of determining toxic effect on the growth of mycelium was suggested by Bateman (1933).

EXPERIMENTAL RESULTS

In table 1 are shown the concentrations of cycloheximide at which the growth of thirty-three plant pathogens was completely inhibited

TABLE 1

THE *In Vitro* EFFECT OF CYCLOHEXIMIDE UPON THIRTY-THREE PHYTOPATHOGENIC FUNGI

Organisms	Disease	Micrograms per milliliter completely inhibiting growth	
		48 hours	72 hours
Class Phycomycetes			
Order Peronosporales			
Pythium debaryanum	Damping Off	10.0	20.0
Class Ascomycetes			
Order Pezizales			
Sclerotinia fructicola No. 19	Brown Rot of Peach	2.5	5.0
Sclerotinia fructicola B	Brown Rot of Peach	10.0	20.0
Sclerotinia fructicola BTI	Brown Rot of Peach	10.0	20.0
Diplocarpon rosae	Black Spot of Rose	5.0	10.0
Order Myriangiales			
Elsinoe veneta	Anthracnose of *Rubus*	20.0	40.0
Order Hypocreales			
Gibberella saubinetii	Barley Scab	10.0	20.0
Order Sphaeriales			
Physalospora tucumanensis L.S.	Red Rot of Sugar Cane	5.0	10.0
Physalospora tucumanensis D.S.	Red Rot of Sugar Cane	5.0	10.0
Diaporthe citri	Canker	2.5	2.5
Endothia parasitica	Chestnut Blight	5.0	20.0
Gnomonia veneta	Anthracnose of Sycamore	10.0	20.0
Gnomonia leptostyla	Anthracnose of Hickory	10.0	20.0
Guignardia aesculi	Leaf Blotch of Horse Chestnut	5.0	10.0
Venturia inaequalis 365-4	Apple Scab	5.0	10.0
Class Basidiomycetes			
Order Ustilaginales			
Ustilago tritici	Loose Smut of Wheat	0.062	0.125
Ustilago zeae	Common Smut of Corn	2.5	10.0
Fungi Imperfecti			
Cercospora apii	Leaf Spot of Celery	20.0	20.0
Heterosporium iridis	Leaf Blight of Iris	2.5	5.0
Cladosporium fulvum	Leaf Mold of Tomato	0.125	0.25
Cladosporium paeoniae	Leaf Mold of Peony	5.0	10.0
Macrosporium sarcinaeforme	Leaf Spot of Clover	10.0	20.0
Alternaria solani	Early Blight of Potato	20.0	40.0
Alternaria solani BTI	Early Blight of Potato	10.0	10.0
Alternaria oleracea BTI		10.0	20.0
Ramularia pastinaceae	Leaf Spot of Parsnip	40.0	100.0
Diplodia zeae	Diplodia Disease of Corn	1.25	1.25
Colletotrichum lagenarium	Anthracnose of Cucurbits	2.5	10.0
Colletotrichum phomoides	Anthracnose of Tomato	10.0	10.0
Fusarium lycopersci	Tomato Wilt	40.0	100 0
Sphaeropsis elysii	Leaf Spot of Black Pine	0.062	0.125
Sclerotium rolfsii	Stem Rot	1.25	2.5
Rhizoctonia sp.	Dollar Spot	1.25	1.25

after 48 and 72 hours of incubation. With the exception of the three non-sporulating fungi mentioned above, the inhibition observed was predominantly that of spore germination. It will be noted that in almost every case a concentration of cycloheximide that prevented germination of spores after 48 hours was insufficient to do so after 72 hours. In an experiment carried to 92 hours there was growth at still higher concentrations than at 72 hours. At any given time partial inhibition of growth could be observed over a range of two to sixteen fold differences in dosage. The growth of all species and strains of fungi tested was completely inhibited after forty-eight hours of incubation by a concentration of cycloheximide of 40 micrograms per milliliter or less. Of the thirty-three organisms, two were inhibited by 40, three by 20, ten by 10, seven by 5, five by 2.5, three by 1.25, and three by less than 1 microgram of cycloheximide per milliliter.

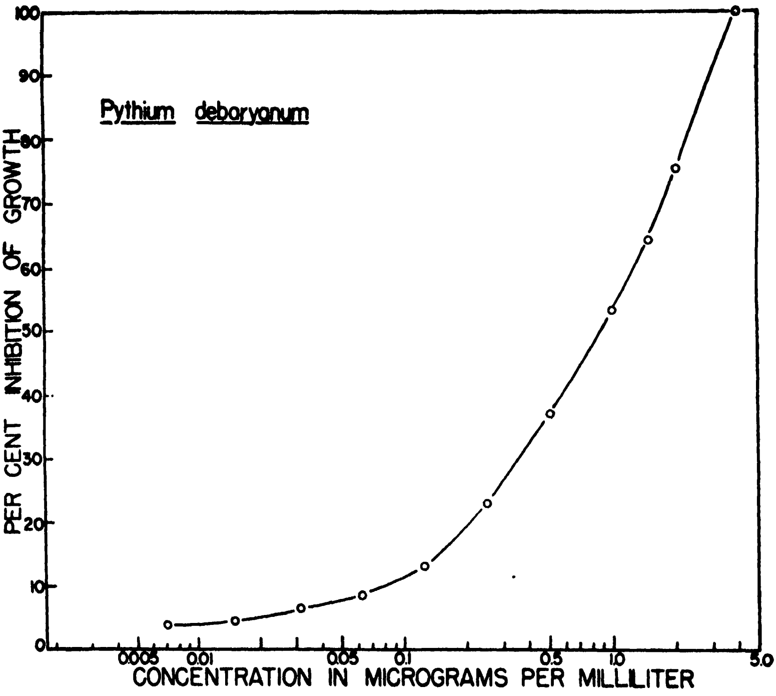

FIG. 1. Dosage-response curve of *Pythium debaryanum* to cycloheximide.

The dosage-response curve of *Pythium debaryanum* to cycloheximide is shown by figure 1 in which the dosage has been plotted on a logarithmic scale against the percentage of mycelial inhibition. When plotted in this manner, the toxicity curve is not a straight line; nor is a straight line obtained when probit response is plotted against logarithm of dosage. The dosage response curve of *P. debaryanum* on logarithmic probability paper has a double slope, curvature concave upwards, and resembles the toxicity curve found by McCallan *et al.* (1941) for Bordeaux Mixture against *Glomerella cingulata.*

DISCUSSION AND CONCLUSIONS

Cycloheximide has an inhibitory effect against a wide variety of phytopathogens including one order of Phycomycetes, four orders of Ascomycetes, one order of Basidiomycetes and twelve genera of the Fungi Imperfecti. In view of its widespread activity against fungi pathogenic to plants, it is surprising that cycloheximide apparently is ineffective against the filamentous fungi that parasitize animals.

Including cycloheximide, approximately twenty-two antibiotic substances have been described in the literature as possessing antifungal properties. Of these only one other than cycloheximide has been extensively tested against phytopathogenic fungi. An impure preparation of antimycin, an antibiotic produced by *Streptomyces* sp., inhibited the growth of eight different species of fungi pathogenic to plants in concentrations of less than two micrograms per milliliter and five other species in concentrations from three to two hundred and fifty micrograms per ml. (Leben and Keitt, 1948). Pure antimycin may be expected to be more highly active *in vitro* than cycloheximide.

The *in vitro* activity of cycloheximide against fungi pathogenic to plants described in this paper is merely an indication of its possible usefulness in the control of plant disease. In addition to determining the toxicity of cycloheximide to pathogenic fungi under field conditions, it will also be necessary to know the toxicity of cycloheximide to the affected plants. Experiments directed to this end have been begun (Vaughn *et al.*, 1949).

SUMMARY

The antibiotic cycloheximide inhibited the growth *in vitro* of thirty-three species and strains of phytopathogenic fungi within a range of concentration varying from 0.125 to 100.0 micrograms per milliliter. The effect of cycloheximide upon the mycelial growth of *Pythium debaryanum* was determined and the dosage response curve plotted.

ACKNOWLEDGMENT

The assistance and interest of Dr. Ralph Lewis in the collection and identification of the pathogenic fungi is gratefully acknowledged.

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THE GENUS MARSSONINA ON QUERCUS AND CASTANEA

PAUL L. LENTZ *

(WITH 1 FIGURE)

A specimen of a fungus causing oak leaf spots was recently sent for determination to the Bureau of Plant Industry by Professor A. A. Dunlap of the Department of Plant Pathology, A. & M. College of Texas. The spots seemed to be caused by a species of *Marssonina*, but the organism was not *M. martini* (Sacc. & Ell.) Magn., the common species on oak. Eventually it was learned that some specimens similar to this fungus had previously been placed in the mycological collections of this Bureau as *Gloeosporium septorioides* Sacc., and that others had mistakenly been determined as *M. martini.* Examination of additional specimens and a search through the mycological literature revealed much confusion concerning this organism. Since this fungus is apparently of rather frequent appearance on oak, the following discussion is presented with the hope that some of the confusion may be eliminated and that attention may be directed to the fact that there are two species of *Marssonina* common on oak in the United States.

In 1884, Winter described *Marsonia quercina* from a specimen collected in Missouri by Demetrio. The fungus sent from Texas by Professor Dunlap belongs to the same species as that described by Winter. The original description by Winter is clear and adequate, except for the necessity of extending the size range of the conidia and other slight modifications.

The fungus described as *Marsonia quercina* causes spots on oak leaves similar to those caused by *M. martini.* The margin may be somewhat more definite and of a slightly darker reddish-brown color than the margin of the spot caused by *M. martini,* but this is not always true. The most reliable macroscopic character that may be used for distinguishing the present fungus from *M. martini* is the host upon which it is found. Of 45 specimens of *M. martini* examined during this study, all were on leaves of species belonging to the white oak group. (*Cf. Marsonia quercus* Pk. below.) The 38 specimens of Winter's *Marsonia quercina* studied all occurred on leaves of species belonging to the black oak group.

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Marssonia ochroleuca (Berk. & Curt. ex Pk.) Humph. ex Seym. (*cf.* below) on *Castanea* resembles the "black" oak fungus even more closely than does *M. martini*, and it is surprising that specimens from oak do not seem to have been given the name of the chestnut fungus. Both *Marssonia ochroleuca* and the "black" oak fungus differ from *M. martini* by having longer, more slender conidia which are not constricted at the septum. The acervuli of the chestnut fungus seem to be somewhat more shallow than those of the "black" oak fungus, but this is not constant. Although the chestnut fungus and the "black" oak fungus are very much alike in every respect, with only vague differences easily accounted for by the difference in host, the two are nevertheless here treated as separate species. This is in keeping with the recognition of the usual association of leaf spot fungi with narrowly restricted hosts and also with tradition, as indicated by the failure of other workers to identify the "black" oak fungus as *Marssonia ochroleuca*.

Part of the confusion concerning the identity of the present fungus no doubt results from the variable nature of the acervulus, an unfortunate phenomenon so far as taxonomy is concerned. It may vary from a sub-sphere to a saucer in form. At maturity it is usually opened broadly and lacks any conspicuously defined wall of the type which would permit it to be considered a pycnidium. Usually the fungus elements at the base of the conidiophores form a thin basal stratum of more or less isodiametric, hyaline and inconspicuous cells, although occasionally there may be an increase in density and definiteness and a slight darkening in some areas, particularly at the base of the fructification. While the acervulus of *M. martini* is confined to the region just beneath the epidermal layer, the fructification of the "black" oak fungus may be confined to this area or may form a deep cavity in the leaf tissue, hypophyllous fructifications frequently forming a cavity extending to the lower margin of the palisade layer of leaf tissue. At maturity the fructification is usually broadly open, and the conidia are freed by splitting of the leaf epidermis over the acervulus.

An additional source of confusion concerning this fungus has been the failure of some workers to see the single septum of the conidium, a structure which frequently is difficult to see by the usual staining methods. By the following method, used during this study, it may be observed with ease: A bit of the acervulus was obtained by use of a needle filed down to a chisel-like edge at the tip. This particle was placed on a slide, moistened with alcohol, swelled with 2 per cent KOH solution, stained with phloxine, and crushed beneath a cover slip by gentle tapping on the latter. Measurements of conidia were then made and were found to differ very little if at all from measurements of conidia well moistened in water. The septum could usually be discerned with some difficulty when the conidia were being measured. Following this examination, the cover slip was removed and a drop of glycerine acidulated with acetic acid was placed on the bit of crushed acervulus. The cover slip was replaced, and examination of the conidium then revealed that the cytoplasm had pulled away slightly from the septum, and that the latter could be seen distinctly.

During this study, it was found that specimens of the "black" oak leaf fungus had been placed in the mycological collections of this station under the following designations: *Marsonia* * *quercina* Wint., *Marssonia quercina* Wint. var. *major* Overh., *Gloeosporium septorioides* Sacc., *Marssonina quercus* (Pk.) Magn., *Marssonina martini* (Sacc. & Ell.) Magn., *Septoria quercicola* Sacc., *Septoria quercus* Thuem., and *Cylindrosporium microspilum* Sacc. & Wint. Of these, the first three represent synonyms of the new combination presented below for the "black" oak fungus. The others are misdeterminations.

To make the matter clear, the following list is presented of species which examination of specimens and/or consultation of literature has shown to be entirely distinct from the fungus occurring on leaves of trees in the black oak group: *Marssonina martini* (Sacc. & Ell.) Magn., *Marssonina quercus* (Pk.) Magn., *Marsonia matteiana* Sacc., *Phleospora hanseni* Bub., *Ascochyta quercus* Sacc. &

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* *Marsonia* and *Marssonia* are earlier, now invalid, names for the present genus *Marssonina*.

Speg., *Ascochyta quercuum* (Cke.) Sacc., *Cylindrosporium microspilum* Sacc. & Wint., *Cylindrosporium quercus* Sorok., *Cylindrosporium quercinum* J. Carter, *Septoria quercicola* Sacc., *Septoria quercina* Desm., *Septoria quercus* Fckl., *Septoria quercus* Thuem., *Septoria ocellata* (Lev.?) Sacc., and *Septoria neglecta* Earle.

A portion of the type collection of *Marsonia quercus* Pk. was studied through the courtesy of Dr. H. D. House of the New York State Museum. In a letter accompanying the specimen, Dr. House expressed doubt that the host was really *Quercus ilicifolia* Wang., as stated in Peck's (**1885**) original description of the species. He suggested that the type material was actually collected from two hosts: *Q. rehderi* Trel. and *Q. prinoides* Willd. Examination of a portion of the type collection on leaves of what appear to be two different species of oak shows *Marsonia quercus* Pk. to be identical with *M. martini* (Sacc. & Ell.) Magn. Thus *Marssonina quercus* (Pk.) Magn. is a synonym of *M. martini* (Sacc. & Ell.) Magn. Previous experience, noted above, of finding *M. martini* only on leaves of species of the white oak group leads to the conclusion that both of the species of oak in the Peck collection must belong to this group as, judging from the configuration of the leaves, they well may.

Magnus (**1906**) has shown that *Marssonia* Fisch. is not valid as the name of a genus of fungi; therefore he changed the name to *Marssonina* Magn. During the study of the fungus known as *Marssonia ochroleuca,* on *Castanea,* it was noted that a transfer to *Marssonina* had apparently never been made. Heald (**1926**, p. 29) has published a photograph of this fungus, beneath which is the caption "Leaf spot (*Marssonina ochroleuca*) of chestnut." There is no further discussion, and this hardly constitutes publication of a new combination. It is probable that Heald merely substituted the new generic term for the old without concerning himself over the fact that the combination had really never been made. *Septoria ochroleuca* was published as a Berk. & Curt. species by Peck (**1873**). Berkeley (**1874**) later published the same species, but his description is antedated by Peck's. On specimens 173a and 173b of Seymour and Earle's Economic Fungi is the designation "*Marsonia ochroleuca* (B. & C.) Humphrey in litt." In Seymour's Host Index of the North American Fungi (**1929**), the species is

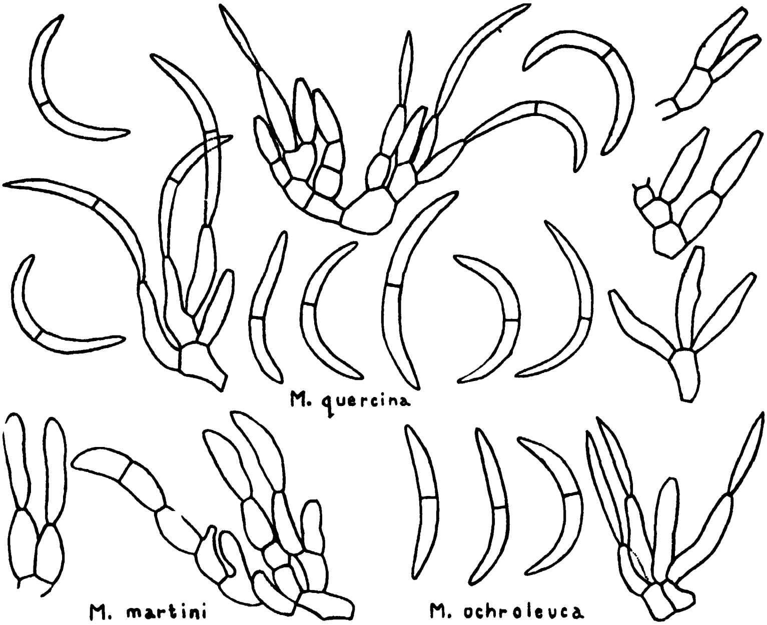

FIG. 1. Conidiophores and conidia of *Marssonina quercina* (top), *M. martini* (bottom, left), and *M. ochroleuca* (bottom, right). Magnification, × 1000.

included, among other synonyms, as "*Marssonia ochroleuca* (B. & C.) J. E. Humphrey." For this fungus, the following new combination is proposed: **Marssonina ochroleuca** (Berk & Curt. ex Peck) comb. nov.

In addition, the following new combination, with synonymy and description, is presented for the fungus which occurs on species of the black oak group:

Marssonina quercina (Wint.) comb. nov.

Marsonia quercina Wint. Hedw. **23**: 171. 1884; also in Rab.-Wint. Fungi Eur. no. 3085. 1884.
Gloeosporium septorioides Sacc. R. Instituto Veneto Atti. Ser. 6. **3**: 726. 1885.
Gloeosporium septorioides Sacc. var. *major* Ell. & Ev. Jour. Myc. **1**: 111. 1885.
Marssonia quercina Wint. var. *major* Overh. Mycologia **18**: 32. 1926.

Leaf spots amphigenous, few and scattered to numerous and sometimes confluent, orbicular to angular-orbicular, (0.5)1–4.5

(5.5) mm. in diameter, the upper surface dull tan[1] (intermediate between light buff and warm buff of Ridgway), or more rarely faded clay color, the margin usually extremely narrow but conspicuous, chestnut brown, verona brown to warm sepia, or seal brown, with the leaf sometimes discolored up to 2–3 mm. from the margin, the lower surface dull tan to cinnamon buff or faded clay color, with the margin often less conspicuous than that of the upper surface, concolorous with the spot, or more often Prouts brown, snuff brown to bister, or seal brown; the acervulus usually one but occasionally several to the spot, usually hypophyllous but occasionally epiphyllous, subglobose with a broad opening to concave or nearly plane, covered by the leaf epidermis, then opening by splitting of the latter, 50–150 μ from base to apex and 150–250 (390) μ in diameter, lacking a conspicuous pycnidium-like exterior wall, sometimes slightly darkened toward the base, but usually with the conidiophores arising from a thin base of hyaline more or less isodiametric cells; the conidiophores of two to several hyaline cells, unbranched, or more often branched one to three times; the apical, conidium-bearing cells of the conidiophores hyaline, slenderly obclavate, (6.5) 10–13 × (1.5) 2–3 μ, bearing a single conidium at the truncate apex; the conidia hyaline, smooth, acerose to falcate, non- or 1-septate as seen attached to the conidiophore, 1-septate when discharged, not constricted at the septum, (14.5) 16–26 (30) × 1.5–2 (2.5) μ, most often 18–23 × 1.5–2 μ, oozing out of the acervulus in an amber brown mucilage which often forms a thin resinous-appearing coating over the leaf spot when dry.

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[1] With the exception of the terms "reddish-brown," "tan" and "amber brown," the terms used in this paper to describe colors are those used in Ridgway (1912).

PERMANENT STAINED MYCOLOGICAL PREPARATIONS OBTAINED BY SLIDE CULTURE

Roland W. Riddell *

(with 17 figures)

The cultivation of fungi on microscopic slides, either by van Tieghem cell or by culture chamber methods, is a routine laboratory procedure. All such procedures commonly employed have certain inherent disadvantages and limitations. These include the amount of time and labor involved in preparation, and the difficulty due to the presence of culture medium when staining and making permanent preparations. These objections are largely obviated by a method to be described below which has, in addition, the asset of providing adequate aeration during culture. The method is superior to all that have been tested, and is quite similar to one used at the London School of Hygiene and Tropical Medicine. It is presented herewith since it is believed that it should be widely employed in mycological laboratories.

About 10 ml. of any chosen agar medium is melted and poured into a sterile 9 cm. Petri dish to form a layer 2 mm. deep. After the medium solidifies, preferably by refrigerator cooling, 1 cm. squares are ruled out over the whole plate using a sterile dissecting needle and a flamed glass rod (fig. 1). This is done rapidly to reduce chances of contamination while the lid is displaced. Using sterile forceps, a flamed microscopic slide is placed under cover of a Petri dish lid, the inside of which was also flamed. A flamed cover slip is placed upon this slide to overlap at one end. An agar square is now lifted out of the Petri dish with precautions to prevent contaminations (fig. 2), and is transferred rapidly to the center of the cooled slide (fig. 3). A needlepoint inoculum of spores, or a small bit of mycelium, is placed at the center of each

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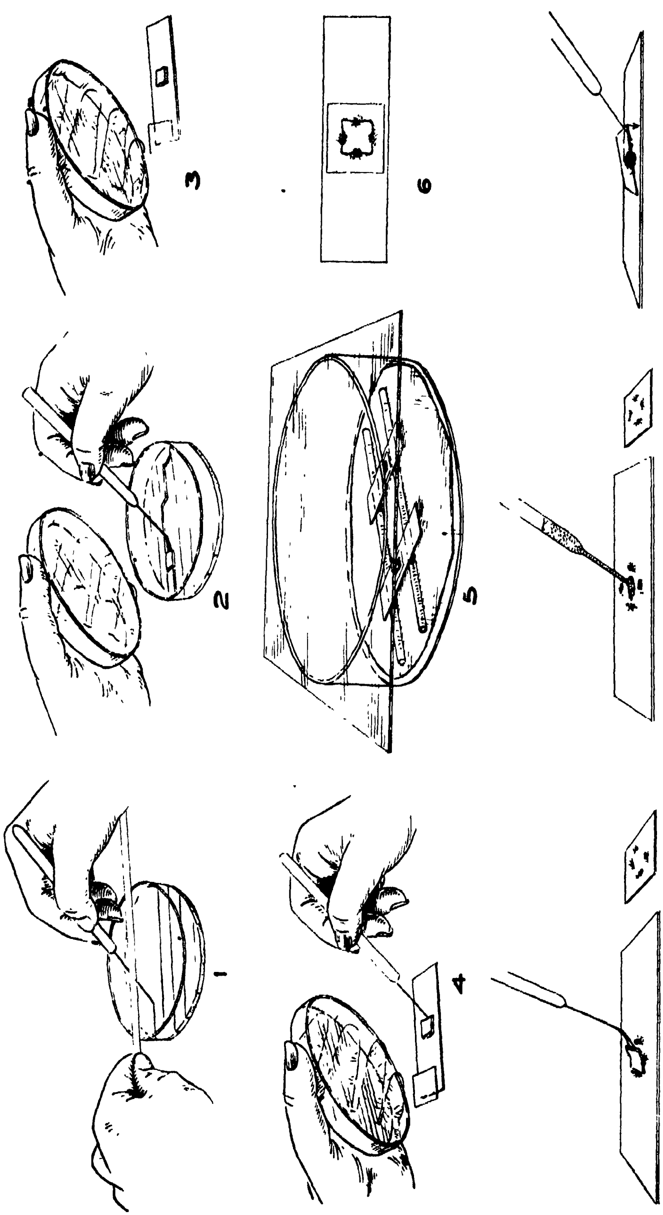

FIGS. 1–9. Technique for making slide cultures.

edge of the agar block over the whole 2 mm. depth at this point (FIG. 4). With sterile forceps the cover slip is placed centrally upon the upper surface of the agar square, and the slide is then transferred to a moist chamber (FIG. 5). Care is taken that the cover slip is not moved during growth of the fungus as when changing from the 16 mm. to the 4 mm. objective in microscopic inspections.

The earliest suitable time before excessive sporulation occurs is chosen to terminate growth. It is rarely necessary to allow growth to extend beyond 3 mm. from the cover slip edge, and often growths of about 1 mm. wide give the best results (FIG. 6). The cover slip is lifted vertically from the agar block without twisting movement and is placed aside, culture uppermost. Beginning at one corner the lifter is gently inserted under the agar block so as to lift it completely off the slide (FIG. 7). Thereby the agar and most of the fungus growth are discarded and all that remain on both slide and cover slip are four areas of growth joined by scanty lines of mycelium. A drop of 95 per cent ethyl alcohol is applied to the center of each of the rings of growth and is allowed to flow outwards to wet the fungus (FIG. 8). Just before it has completely evaporated a drop of lactophenol blue [1] is applied as was the alcohol. A clean cover slip is gently lowered upon the slide culture to spread the dye outwards over the damp mycelium (FIG. 9). Similarly a clean slide is applied to the cover slip preparation and is then quickly inverted. The slides are left undisturbed overnight after which any dye expressed is absorbed by strips of blotting paper applied to the cover slip edges. Fixation of the cover slips can now be obtained by application of colored nail lacquer to the corners. When this has dried, more lacquer is used to seal off the cover slip edges and, when dry, it may be trimmed with a razor blade. Two permanent stained preparations are thus made from each slide culture.

The agar concentration (*e.g.*, about 1.5 per cent Bacto-agar in Sabouraud's medium) should be suitable to allow a square to be lifted out cleanly without fraction. A lifter can be made by ham-

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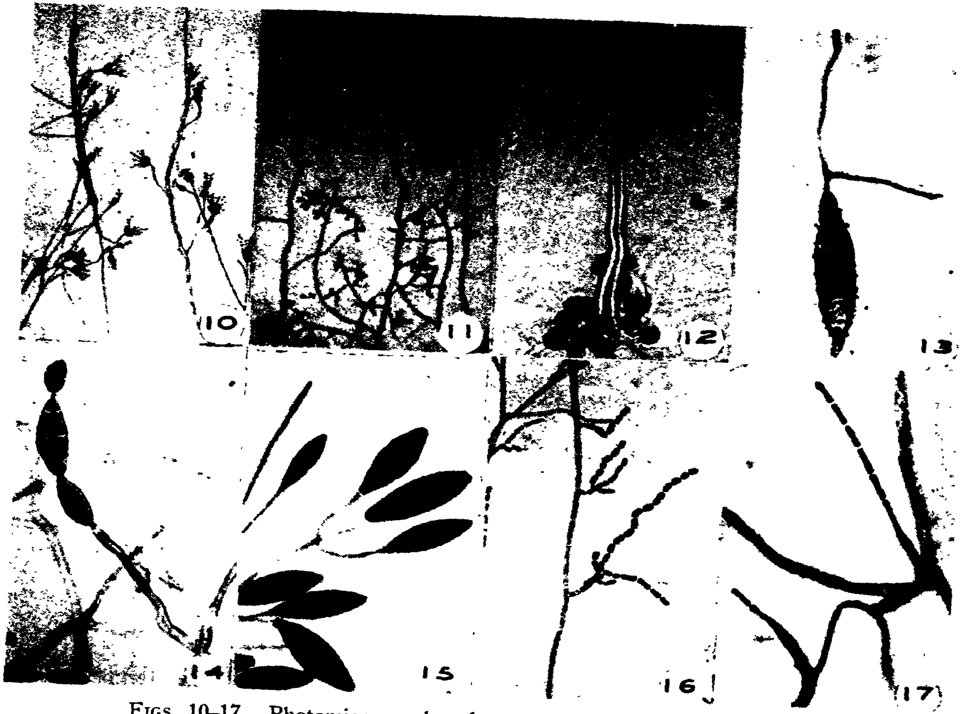
[1] Lactophenol blue: phenol crystals 20 gm., lactic acid 20 cc., glycerol 40 cc., distilled water 20 cc. Dissolve by gently heating and add 0.05 gm. cotton blue (methyl blue).



FIGS. 10–17. Photomicrographs of various fungi from slide cultures.

mering out the red-hot end of a dissecting needle bent to an obtuse angle. The Petri dish of unused agar blocks may be stored in the refrigerator. If the agar block is too thick little growth becomes attached to the glass, whereas if too thin it shrinks during the period of fungus growth and becomes difficult to remove. The moist chamber is constructed from a glass dish in which are laid two layers of paper towelling soaked in 20 per cent glycerol in water, and the slide cultures are supported above the paper by glass rods. The rim of the dish is sealed with vaseline and a glass plate is laid upon it. The chamber need not be sterile as is demonstrated by the rarity of contaminants in the cultures. The glycerol is incorporated to prevent "fogging" of the slides. It may be necessary to add more glycerol solution to the paper during culture but excessive wetting is avoided. There should be no marked shrinkage of agar during incubation. The application of alcohol to the fungus prevents inclusion of air bubbles and facilitates staining, but an excess present when the dye is added gives irregular results. The size of the lactophenol blue drop is adjusted so as just to fill a cover slip area. It is desirable that a centrifugal movement of alcohol and dye over the area occupied by the fungus is achieved, and as little movement of the cover slip as possible. Where sporulation is excessive the cover slip preparation is the better of the two, as it is relatively free from deposited spores. A *Penicillium* species is a suitable organism with which to practice the technique.

The method provides a simple means of observing aerial and submerged mycelial growth of fungus and developing fruiting structures in situ. Permanent stained preparations are made which demonstrate general fungus distribution (FIGS. 10 and 11), stages of spore germination (FIGS. 12 and 13), and the development of fruiting structures (FIGS. 14 and 15). It is at the moment being used to investigate the effects of chemicals and antibiotics present in the medium upon cultural morphology (FIG. 16). Conidial apparatus may be studied in detail (FIG. 17). It provides a rapid routine diagnostic aid to supplement observation made by teasing apart preparations on a slide. In medical mycology, it is possible to use for demonstration slides small portions of infected hair or skin scales as inocula.

DESCRIPTION OF FIGURES

Photomicrographs of various fungi from slide cultures stained with lactophenol blue: (10) *Penicillium* sp. (11) *Neurospora sitophila.* (12) *Aspergillus* sp.—germination and sporulation. (13) *Microsporum canis*—macroconidium germination. (14) *Alternaria* sp.—conidium formation. (15) *Microsporum gypseum*—stages in macroconidium development. (16) *Polyporus versicolor*—Oidium production on monokaryotic mycelium. (17) *Endoconidiophora* sp.—conidiophores.

A NEW GENUS OF THE CHOANEPHORACEAE

LELAND SHANOR, ADRIAN W. POITRAS, AND R. K. BENJAMIN

(WITH 12 FIGURES)

The family Choanephoraceae is one of the smaller families of the order Mucorales but one of considerable interest because certain of its species are parasitic on higher plants and, within the family in the various genera, the spores may be formed in Mucor-like sporangia, in sporangioles, or as monosporous sporangioles which are usually referred to as conidia. The genera are separated primarily on a combination of the different sporulating structures produced and from two to as many as six genera are included in the family by different authors (Fitzpatrick, **1930**; Zycha, **1935**; Naumov, **1939**). The six genera included by Zycha (**1935**) are: *Blakeslea* Thaxter, *Choanephora* Currey, *Cunninghamella* Matruchot, *Rhopalomyces* Corda, *Thamnocephalis* Blakeslee, and *Sigmoideomyces* Thaxter. In *Blakeslea* many-spored sporangia and sporangioles are produced but no conidia; in *Choanephora* many-spored sporangia and conidia are formed but no sporangioles; in *Cunninghamella, Rhopalomyces, Thamnocephalis.* and *Sigmoideomyces* only conidia are known. Naumov (**1939**) includes only *Choanephora* and *Blakeslea* in the Choänephoraceae and segregates *Cunninghamella, Sigmoideomyces* and *Thamnocephalis* in a separate family, the Cunninghamellaceae. Zygospores in *Blakeslea* and *Choanephora,* where known, are formed between the tips of twining branches and the species are heterothallic. Zygospores in *Cunninghamella,* where known, are formed more as are those of *Mucor* rather than between tips of twining branches. Zygospores in *Rhopalomyces,* in *Thamnocephalis,* and in *Sigmoideomyces* are unknown, a fact which has influenced some students to exclude these genera from the Mucorales.

Early in March 1949 there appeared on a collection of rabbit dung taken in Brownfield Woods near Urbana, Illinois, a very interesting mucoraceous fungus which formed zygospores of the Mucor type in great abundance and produced asexual spores in sporangioles which arise on the end of long stalks from inflated tips of erect hyphae characteristic of the Choanephoraceae. This fungus was isolated in single spore culture and has been grown since on a number of culture media. Sporangiole formation on a capitate vesicle on erect sporangiophores has been the only type of asexual reproduction obtained. In the way in which it reproduces asexually, this fungus appears most closely related to *Blakeslea* and *Choanephora* but cannot be included in either genus as defined at present, and it differs strikingly from either in the way in which zygospores are formed. The type species, which at present represents the only one known in this new genus, is homothallic. The genus is being named in honor of Professor William Chambers Coker.

Cokeromyces Shanor, gen. nov.*

Sporangiolis globosis natis e stipitibus quae affatim nascuntur in summis sporangiophorum tumoribus terminalibus et capitatis; sporis nigris quae minime sunt striatae nec appendiculas habent radiatus; sporangiis aut conidiis nullis. Zygosporae inter ramos conexivos nascuntur eodem modo ac zygosporae Mucoris vel Cunninghamellae, et non inter tortilium ramorum apices ut Blakesleae vel Choanephorae zygosporae.

Sporangioles produced abundantly on stalks over the surface of terminal capitate swellings of sporangiophores; spores dark and not possessing radiating delicate cilium-like appendages; sporangia and conidia absent. Zygospores produced between short, relatively straight copulating branches as in *Mucor* or *Cunninghamella* and not between tips of twining branches as in *Blakeslea* or *Choanephora.*

Cokeromyces recurvatus Poitras, sp. nov. (Figs. 1–12)

Sporangiolis globosis quae nascuntur in cacuminibus longorum stipitum qui e vesiculis terminalibus crescunt sporangiophorum ramis plerumque carentium quae se usque ad 500 μ e substramine porrigunt; sporangiophoris plerisque 9 μ in diametro; vesiculis terminalibus 12.6–31.5 μ (vulgo 21 μ) in diametro; sporangiolorum stipitibus 60–120 μ (vulgo 80 μ) longis, 2.2 μ fere in diametro, recurvatis atque tortilibus cum maturuerunt; sporangiolis globosis 8.4–12.6 μ (vulgo 10 μ) in diametro, parietes foris leves sed intus reticulatos habentibus,

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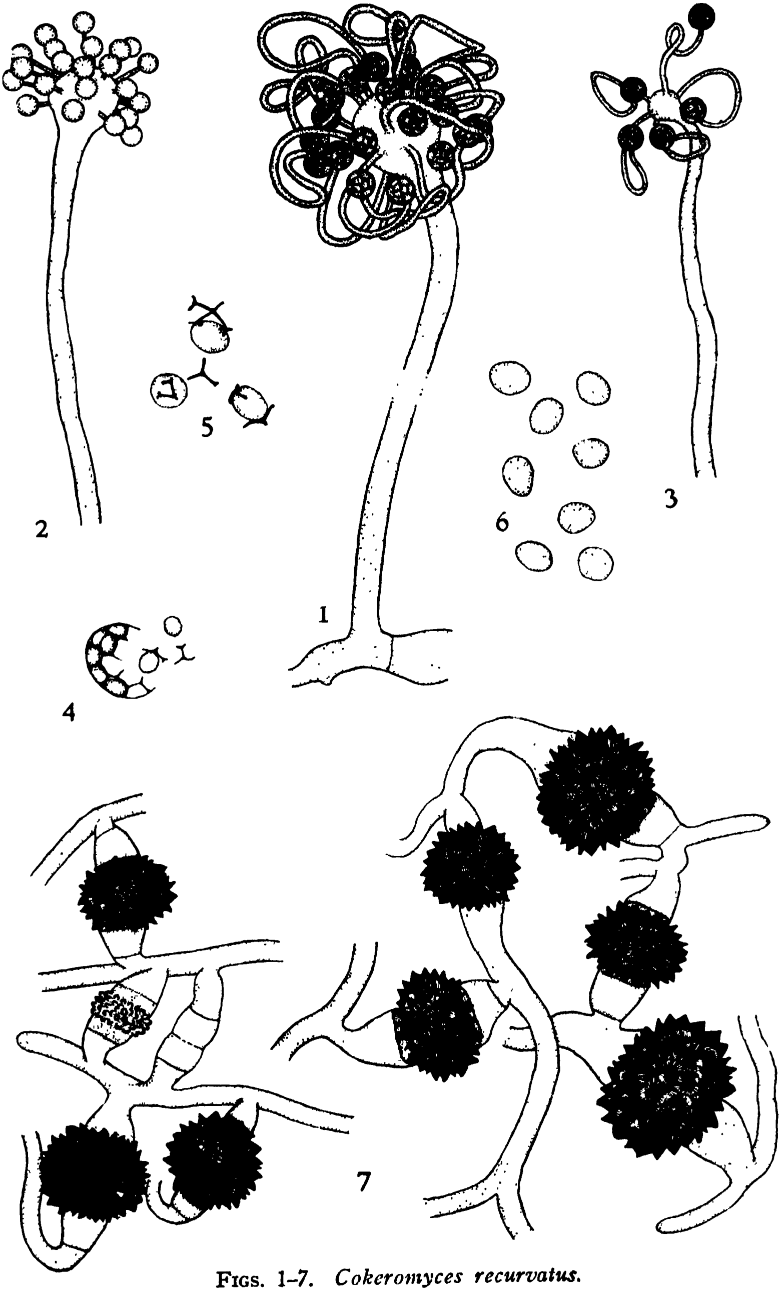
* We are indebted to Professor Revilo Oliver, Classics Department, for the preparation of Latin diagnoses.



FIGS. 1–7. *Cokeromyces recurvatus.*

5–30 vel plura e vesicula terminali nascuntur; sporis non unius magnitudinis ovoidibus vel incompositis, leves parietes habentibus, 2.5–4.5 μ, quorum duodecim vel plura usque ad viginti sporangiolum fert. Sporangia et conidia plane desunt. Zygosporae quae affatim nascuntur sive inter ramos conexivos e hyphis superficialibus crescentes sive ex zygosporarum iam maturatarum suspensoribus, hispidos habent parietes e quibus prominentia retusa vel teretia vel acuta usque ad 4 μ se porrigunt. Hae zygosporae globosae fuscae et rubidae sunt cum maturuerunt, 33.5–54.5 μ (vulgo 43–45 μ) in diametro; homothallicae.

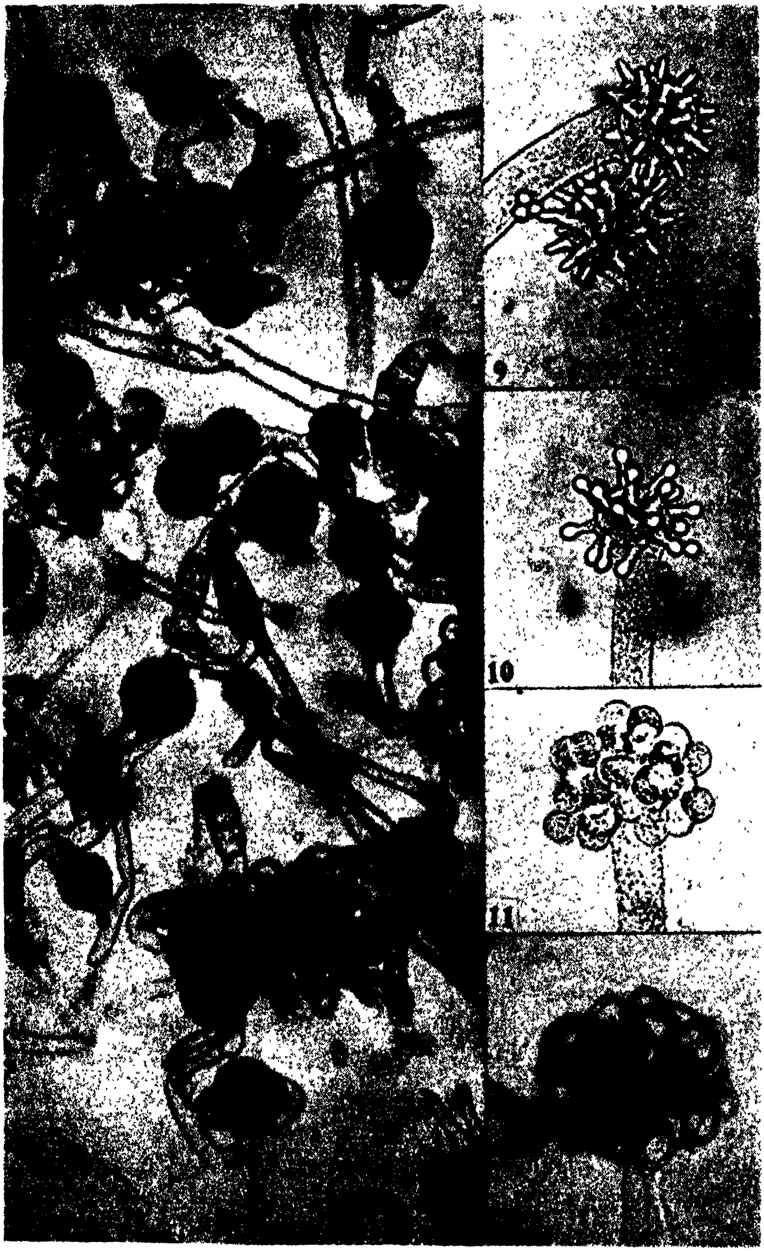
Mycelium rather slow-growing on culture media, producing reproductive structures very quickly; sporangioles formed at tips of long recurved stalks which arise from a terminal vesicle of usually unbranched sporangiophores which extend up to about 500 μ above substrate, usually about 325 μ high; average diameter of sporangiophores about 9 μ; terminal vesicles 12.6–31.5 μ in diameter, usually about 21 μ; sporangiole stalks recurved and twisting when mature, variable in length, 60–120 μ long, usually about 80 μ, about 2.2 μ in diameter with wall thicker and darker than that of the vesicle; sporangioles essentially globose, 8.4–12.6 μ in diameter, usually about 10 μ, outer wall smooth, inner wall surface often reticulate because of a deposit, 5–30 or more formed from each vesicle, usually about 20–22; spores not uniform in size, ovoid to somewhat irregular, commonly measuring about 2.5 by 4.5 μ, not striate, smooth-walled, except outer ones in sporangioles which may have a reticulate deposit on outer surface, usually about 12–20 per sporangiole. Conidia and many-spored sporangia absent. Zygospores produced in great abundance between copulating branches which arise usually from superficial hyphae, sometimes from outgrowths of sporangiophores, and very often from suspensors of more mature zygospores, walls rough because of prominent, variable, blunt, rounded or sharply pointed projections which are up to 4 μ in length; zygospores globose, brown when mature, measuring 33.5–54.5 μ in diameter including projections, usually about 43–45 μ; homothallic.

Developed on rabbit dung collected in Brownfield Woods, Urbana, Illinois, March 17, 1949. Type material deposited in the Mycological Collections of the University of Illinois Herbarium, Mycological Collections No. 20849.

Growth on the original rabbit dung was very profuse, and other sporulating fungi were almost completely absent. Transfers to potato dextrose and to Blakeslee's No. 230 media were made with little difficulty and single spore cultures were subsequently made from these. This fungus grows more slowly on agar and with much less cottony aerial mycelium than any isolate of *Choanephora* or of *Blakeslea* we have examined and such cultures are conspicuously zonate. In the dark zones, sporangiophores bearing sporangioles are formed in abundance but are very rare or completely lacking in the lighter zones. The relative abundance of zygospores in the lighter and darker bands is essentially uniform. Mature sporangioles are distinctly blacker than mature zygospores. The factor or factors controlling this growth pattern have not been completely determined but this seems to be influenced primarily by the effect of light on sporangiophore production. Walls of aerial hyphae, but especially those of sporangiophores, are conspicuously spotted with small hyaline crystals.

Sporangiophores arise from submerged hyphae, are relatively straight or somewhat curved and are terminated by a definite swelling (FIGS. 1, 2, 3, 9, 10, 11 and 12). Before this vesicle has enlarged very conspicuously, the initials of the sporangiole stalks appear. At first these are straight and the initial of the sporangiole itself soon arises as a swelling at the tip (FIGS. 2 and 10). During the period of enlargement of the sporangiole until it reaches almost its mature dimensions, very little elongation of the sporangiole stalk takes place (FIGS. 2, 10, and 11). At approximately the time a cross wall forms to delimit the sporangiole, the sporangiole stalk begins to elongate rapidly, recurving toward the vesicle (FIGS. 1, 3, and 12). Maturation of sporangioles appears to occur after the stalks have completed their elongation.

When sporangioles are crushed an interesting feature of this fungus can be observed that is otherwise likely to be overlooked. During the formation and maturation of spores, there is deposited against the sporangiole wall between the outer layer of spores thin bands of a darkly pigmented substance that sometimes remains attached to the wall to form a reticulation or in other instances may adhere to the layer of spores next to the wall (FIGS. 4 and 5). These delicate fibrils are readily separable from the spores or sporangiole wall on which they may be found when sporangioles are crushed in water, and so could not be regarded as part of the wall, either of spores or of sporangioles. This material appears rather to be clearly a residue from sporangiole protoplasm after spores have



FIGS. 8–12. *Cokeromyces recurvatus.*

been delimited. The spores themselves (FIG. 6) are smooth-walled with no trace of any striations or delicate radiating appendages at the ends, are not uniform in size and are quite variable in shape, usually ovoid to elliptical.

Zygospores (FIGS. 7 and 8) have been formed in great profusion on all types of culture media on which this fungus has been grown. Their abundance is comparable to that observed in cultures of *Zygorhynchus vuillemini* or of *Z. heterogamus* when grown on the same media. They usually form just above the surface of the culture medium, either between branches from hyphae that have just emerged, between branches from older more extensively developed hyphae, occasionally between branches of hyphae and branches from sporangiophores, or more frequently from branches from suspensors of more mature zygospores. They are in all respects much more like zygospores of *Cunninghamella* and of most of the genera of the Mucoraceae than like zygospores of *Choanephora* or of *Blakeslea* where the development of zygospores is more like that in species of the Cephalidaceae.

SUMMARY

A mucoraceous fungus belonging to the Choanephoraceae, representing a new genus and species, is described and named *Cokeromyces recurvatus*. The genus *Cokeromyces* is distinguished from *Blakeslea* and *Choanephora* by its more Mucor-like zygospores, its formation of sporangiola only as representing asexual reproductive structures, and by spores that lack radiating appendages and are not striated.

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URBANA

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EXPLANATION OF FIGURES

FIG. 1. Characteristic sporangiophore with numerous sporangioles. × 685. 2. Apical portion of young sporangiophore before much elongation of the sporangiole stalks has occurred. × 685. 3. Apical portion of small sporangiophore bearing only five sporangioles. × 685. 4. Crushed sporangiolum showing the net-like deposit on inner sporangiole wall which frequently becomes detached and clings to spores. × 1580. 5. Three of the outer layer of spores with associated detached deposit. × 2160. 6. Eight spores showing usual appearance. × 2160. 7. Two groups of zygospores in different stages of development. × 685. 8. Photomicrograph of portion of culture showing abundant zygospores in different stages of development and three sporangiophores as seen from above. × 275. 9. Photomicrograph of apical portion of very young sporangiophore. × 525. 10. Photomicrograph of slightly older condition than shown in figure 9. × 465. 11. Photomicrograph of young sporangioles on vesicle shortly before elongation of sporangiole stalks takes place. × 400. 12. Photomicrograph of apical portion of sporangiophore with mature sporangioles having strongly recurved stalks. × 250.

STUDIES ON A PLASMODIOPHORACEOUS PARASITE, OCTOMYXA BREVILEGNIAE

WILLIAM R. PENDERGRASS

(WITH 29 FIGURES)

The monotypic genus *Octomyxa* was established by Couch, Leitner, and Whiffen (**1939**). A second species of *Octomyxa* growing in *Brevilegnia linearis* was found in a soil collection from the City Park, Florence, S. C., in June, 1947. It appeared to be similar to *O. Achlyae* but because of negative growth reactions on *A. glomerata* and differences in the size of the galls, zoosporangia, and resting spores, it was believed to be a new species and given the name, *O. Brevilegniae* (Pendergrass, **1948**). A detailed study of this species has now been made.

MATERIALS AND METHODS

Hemp seeds were used as a culture medium throughout the study. The host and parasite were separated from other fungi by placing a mass of the parasitized hyphae of *Brevilegnia* on a 2 or 3 per cent plain agar plate, then separating the hyphae sufficiently to allow a hypha containing a parasitic plasmodium to be cut out on a block of agar. The agar block was then transferred to one of several large drops of water in a sterile Petri dish. The growth on the agar block was allowed to proceed for twelve to fifteen hours, after which a sterile split hemp seed was placed in the same drop of water in contact with the block. Efforts to grow *O. Brevilegniae* with its host on agar were unsuccessful.

Preliminary cytological studies were made using both whole mounts and sectioned material. As a result, it was found that the nuclei of the developing parasitic plasmodium undergo the "cruciform" or "protomitotic" type of division (FIG. 23). The heterocont, biflagellate nature of the zoospores (FIGS. 14, 26) was demonstrated by killing active zoospores in a drop of water on a slide with fumes of 1% osmic acid and staining with gentian violet. The Löffler flagella staining technique (Couch, **1941**) was also used; but no tinsels were observed.

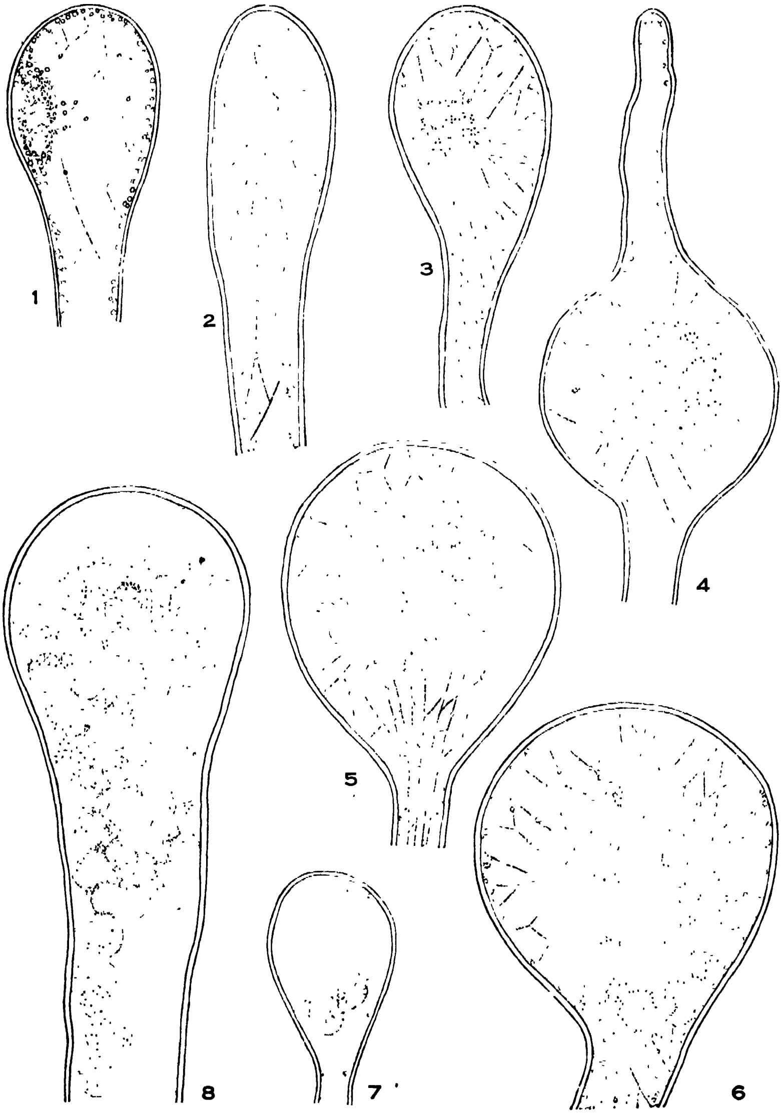
HOST RELATIONSHIPS

Numerous experiments to infect other water molds with *O. Brevilegniae* were carried out. Hyphae containing the parasite in various stages of development were placed in contact with cultures of various water molds likewise in different stages of growth. Hemp seed cultures of *Saprolegnia ferax, S. delica, S. diclina, S. litoralis, S. megasperma, Aplanes treleaseanus, Dictyuchus missouriensis, Isoachlya intermedia, I. itoana, I. unispora, Aphanomyces laevis, Protoachlya hypogyna, Achlya racemosa, A. apiculata, A. colorata, A. caroliniana, A. glomerata, A. crenulata, A. flagellata, A. recurva, A. proliferoides, Thraustotheca primoachlya, T. unisperma, Allomyces javanicus, A. moniliformis, Apodachlya pyrifera* were found to be uninfected after association with the parasite, whereas *Geolegnia inflata* was as susceptible as *B. linearis.*

DEVELOPMENT OF O. BREVILEGNIAE

Zoospores of *O. Brevilegniae* have been seen on the hyphae of the host, but the actual penetration has not been observed even though considerable time was spent in search of such a process. However, it may be safely assumed that the entire zoospore passes into a hypha without first encysting because empty spore cases have never been observed on the hyphae which contained even the youngest stages of plasmodial development. In stained material, one quite frequently finds uninucleate plasmodia back some distance from the hyphal tips. The galls, however, almost always develop at the hyphal tips, carried there by the streaming protoplasm of the host.

This fungus produces two rather remarkable types of plasmodia. At the present time there is no thoroughly satisfactory explanation why one thallus of the parasite forms zoosporangia and another forms resting spores. The study of *Olpidiopsis* by Barrett (**1912**), however, offers the following possible explanation for this condition. The zoosporangia develop while the parasite is still young and plenty of food material is available, whereas the resting spores develop as the host matures and while the food supply is being exhausted. Accordingly, the parasite is provided with facilities for rapid propagation as well as survival during adverse conditions.



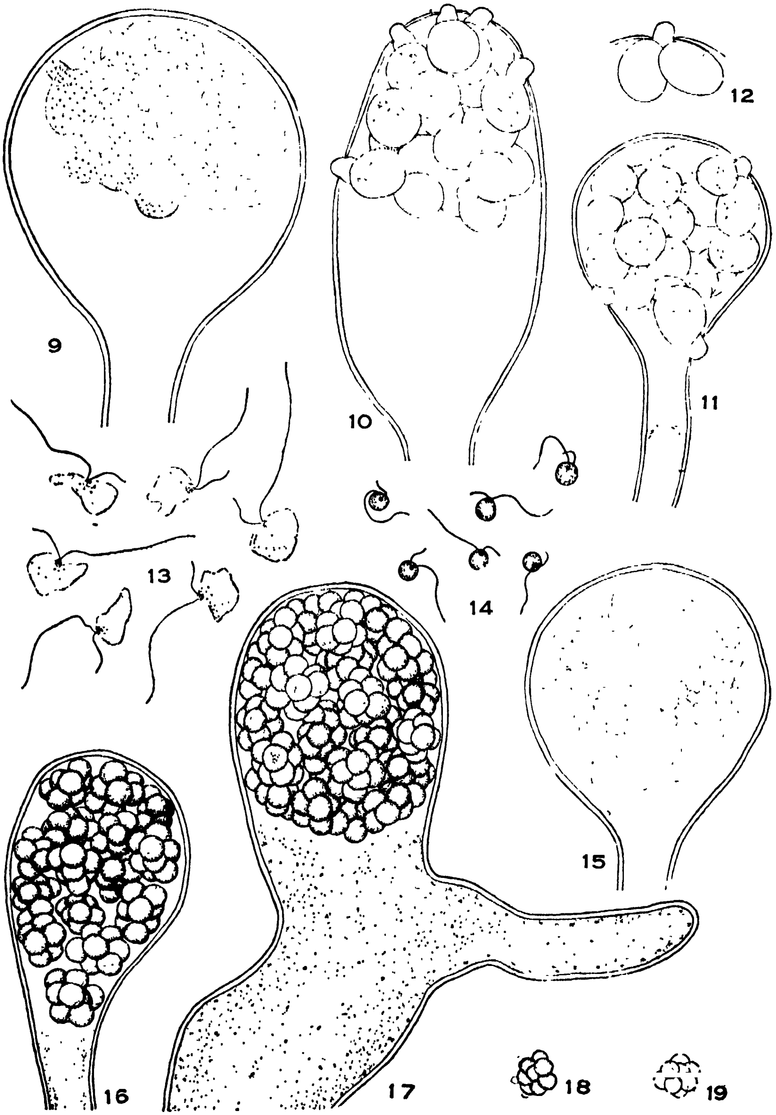
FIGS. 1–8. *Octomyxa Brevilegniae.*

If a new culture is made by cutting off infected hyphae from an old culture and placing them in contact with a piece of hemp seed, new infections of the host will appear in about twenty to thirty hours. Heavily granular hyphae which show a slight swelling at the tips or at a position just behind the tips begin to appear close to the hemp seed. These swellings assume the typical spherical shape after another day of growth. As new infections continue, fully 65 per cent of the host hyphae may show indications of the parasite but later-formed galls are somewhat smaller than the first. Several galls have been seen in each of which there was more than one developing plasmodium. Yet, each plasmodium was clearly independent of the others with no fusion occurring among them.

The study of living plasmodia was made with both water immersion and oil immersion objectives. It was found that the parasite in its early stages takes a position close to the hyphal wall near the tip (FIGS. 1, 21). In this position, there is a flow of globules toward the parasite along cytoplasmic threads of the host, with the result that there is a conspicuous accumulation of these globules around the plasmodium. The globules later become enclosed within the plasmodium. Therefore, it seems obvious that the globules along with the host protoplasm are being drawn into the plasmodium as food. Host protoplasm continues to be drawn into the plasmodium from the expanded hyphal tip as well as from that region of the hypha below the gall. Consequently, the unwalled parasite increases in size (FIG. 3) and soon takes up a position in the center of the gall (FIGS. 4, 22). At the same time, the plasmodium assumes an uneven, reticulate appearance such that lighter areas unequal in size become faintly visible within the rounded mass (FIGS. 5, 24).

Further enlargement of the parasite takes place with the gradual exhaustion of the available host protoplasm. Concurrently with this increase in size, vacuoles may appear within the plasmodium (FIG. 6).

The first evidence of zoosporangial formation is the cleaving of the parasitic thallus into numerous rounded masses which as development proceeds gradually become more distinct (FIGS. 7, 8). Each of these spherical masses becomes a zoosporangium. At this time, the young emergence papillae directed toward the host wall may be noticed on some of the zoosporangia. These papillae contain granules at first but become hyaline, the granules apparently flowing back into the body of the sporangium (figs. 9, 10, 11).



Figs. 9–19. *Octomyxa Brevilegniae*.

Cleavage of the sporangial protoplasm begins as a slight furrowing from the center outward. The spore initials thus have a rounded appearance toward the center of the sporangium with the opposite side conforming with the curvature of the periphery of the sporangium (FIG. 12).

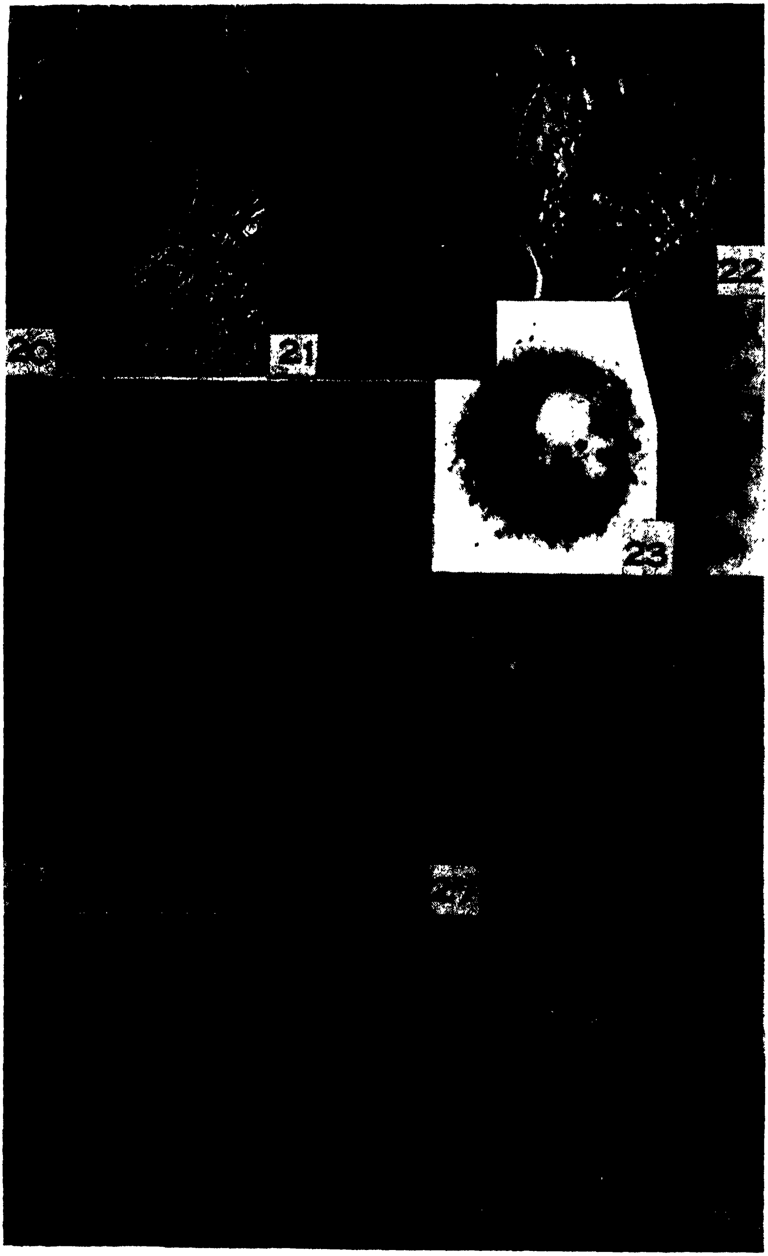
Not all the zoosporangia in any given sorus mature simultaneously. Only the peripheral zoosporangia have papillae. Those situated deeper in the gall are connected to one another and subsequently to the peripheral sporangia by coinciding apertures in their closely appressed sporangial walls. The exit papillae soon penetrate the hyphal walls and finally rupture. Thus the zoospores as they mature have a passageway to the outside. It is not at all uncommon that an exit tube may appear at the juncture of two peripheral sporangia, thus forming the emergence-pore for these two as well as the sporangia beneath them (FIGS. 12, 25). The writer, on several occasions, has seen an exit papilla which gave the appearance of occurring at the juncture of three and even four sporangia. The zoosporangial walls persist after zoospore discharge, disintegrating only after the hyphae of the host have broken down. They give a negative reaction for cellulose.

Flagella are formed on the spores while they are still in the sporangia and considerable movement may be noted prior to their escape. After the sporangia become partly empty, some spores may move from one sporangium to another and then back to the sporangium in which they were formed. The first spores to emerge from the exit papillae appear to be thrust out very violently whereas later ones escape much more slowly. The spores themselves have a peculiar amoeboid posterior end which may bear one or two delicate protoplasmic processes (FIG. 13). After the body of the spore has passed through the exit pore, these delicate posterior processes usually become hung in the papilla whereupon the spore frees itself only after a violent struggle.

Swimming actions of the spores were quite difficult to follow. Some spores exhibited either a rocking motion or a trembling movement whereas others swam in a nearly straight path rotating clockwise on their axes, as viewed under the microscope, with the long posterior flagellum being much less active than the short anterior one.

The development of resting spores takes place in plasmodia very similar to those from which zoosporangia are formed, but it is not easy to distinguish between the two. However, the plasmodia which form resting spores are noticeably darker, due to their much heavier granular contents. Spore formation begins as a rounding up of the unwalled plasmodium into masses which approximate the size of the future resting spores (FIGS. 15, 27). These future spores are rather hard to distinguish and can best be seen on the periphery of the plasmodium as a roughened border. Gradually, the roundness of the spore initials becomes more evident until finally individuals are distinguishable. At this time, there is no indication of an octant grouping and the initials themselves are without definite walls. Once the spores become mature, the groups of eight can be readily seen (FIG. 16). Rarely one finds groups containing seven or nine spores. Although there is no membrane or wall around the groups of eight spores, each group remains intact even after the host wall has disintegrated and the groups are floating in the water.

In view of the importance of determining the ultimate fate of the resting spores, efforts were made to stimulate their germination. Preliminary endeavors consisted simply of cutting from the hemp seed several hyphae containing an appreciable number of cystosori galls, placing the hyphae in a large drop of sterile water on a slide and housing the slide in a damp chamber. Observations were made twice a day for seven days but no indications of germination were noted. Alternate wetting and drying which Jahn (**1905**) found important in the germination of the spores of certain Myxomycetes proved fruitless with the resting spores of *O. Brevilegniae*. In addition, spore galls were subjected first to a temperature of 36° C. for two days, then to a temperature of 0° C. for an equal length of time and vice versa for a second group of galls, but again results in both cases were negative. Further germination studies were made by placing cultures of *B. linearis*, which contained resting spores, into watch glasses, some of which were filled with sterile charcoal water and others with spring water. The watch glasses were then placed in a cabinet with the temperature maintained at 23° C. to 28° C. Nevertheless, the resting spores failed to germinate. Finally, resting spore galls were treated with a number of



FIGS. 20–29. *Octomyxa Brevilegniae.*

decoctions. The first of these decoctions was made by boiling about 50 unparasitized cultures of *B. linearis* in 60 cc. of distilled water for 15 minutes after which the liquid was filtered and sterilized. Drops of the filtrate were placed on a series of slides to which spore galls varying in age from 2 weeks to 2 months were added. Examination, however, showed only negative results for all ages of spores treated. As for the other decoctions, 20 grams of lima bean seedlings (roots and old cotyledons removed), old leaves of rock chestnut oak, new leaves of southern red oak, crushed corn seeds, pine needles, and crushed green peas were each boiled in 60 cc. of distilled water for 15 minutes, filtered, and sterilized. Spores were placed in filtrates of each of the six above-mentioned decoctions as well as in filtrates which were diluted with distilled water in ratios of 3 : 1, 1 : 1, and 1 : 3. The pH of these decoctions ranged from 4.1 for the pine needles to 7.7 for the green pea decoction. No germinations were obtained in any of these decoctions.

Although the actual germination of the resting spores has not been observed, many of the groups of eight spores have been seen upon which wart-like protrusions had formed (FIG. 18). Proof that the resting spores do germinate was supported by the finding of many octads of empty spore cases each with a delicate pore in its wall (FIG. 19). Further circumstantial evidence of germination was obtained from cultures. New growths of the parasite on *B. linearis* were obtained on hemp seeds which had been placed in contact with old (94 days) infected cultures of the host following a change to Petri dishes with fresh water. Consequently, it would appear that spore germination took place and that a period of aging, such as Ziegler (**1948**) found necessary for zygote germination in members of the Saprolegniaceae, is necessary in order to obtain positive results.

SUMMARY

O. Brevilegniae was found to be an obligate parasite on *Brevilegnia linearis* and *Geolegnia inflata*. Infection of the host hypha is by a zoospore. No spore case is left on the hypha. All regions of the hyphae are susceptible to penetration, but the galls are predominantly formed at the hyphal tips. Increase in size of the vegetative thallus is correlated with a reduction in quantity of the host protoplasm in the infected hypha. The plasmodium which is to form zoosporangia rounds up into homogeneous masses. Cleavage of spores from these sporangial initials is from the center outward. Only the peripheral zoosporangia have exit papillae. Those situated deeper in the gall are connected to one another by coinciding apertures in their walls and subsequently to those with papillae. One papilla may appear at the juncture of two or perhaps even three or four peripheral sporangia. Motile zoospores have an amoeboid posterior end as well as a short anterior flagellum and a long posterior flagellum which ends in a whip lash. Resting spores are derived from plasmodia similar to those from which zoosporangia originate, but the former plasmodia are darker in appearance than the latter. These plasmodia are cleaved predominantly into groups of eight spores. Germination of the resting spores seems to be dependent upon an aging period. The empty spore case showed a delicate pore through which the spore emerged.

ACKNOWLEDGMENTS

The writer is grateful to Dr. J. N. Couch for guidance during this study, to Mrs. Alma Beers for arranging the figures, to A. W. Ziegler for cultures of water molds, and to C. D. Beers for information concerning "protomitosis" in the Protozoa.

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EXPLANATION OF FIGURES

All drawings approximately × 735 except figure 23

FIGS. 1–4. Early stages of parasite development. FIG. 5. Plasmodium with reticulate appearance. FIG. 6. Plasmodium with vacuole. FIGS. 7, 8. Young zoosporangia. FIG. 9. Emergence papillae forming. FIGS. 10, 11. Cleavage of spores. FIG. 12. Exit papilla shared by two sporangia. FIG. 13. Sketch of active zoospores. FIG. 14. Stained zoospores with whip lash. FIG. 15. Cleavage of resting spores. FIGS. 16, 17. Resting spore galls. FIG. 18. Octad of resting spores, three of which are excysting. FIG. 19. Empty cysts with pores. FIG. 20. Several galls of *Octomyxa*, × 75. FIG. 21. Parasite close to hyphal wall, × 755. FIG. 22. Plasmodium in center of gall, × 575. FIG. 23. Plasmodium stained with Gram's gentian violet technique. Small photograph of entire plasmodium at left, × 690. Three nuclei in "Protomitotic" division enlarged from original photograph, × 2770. FIG. 24. Zoosporangial plasmodium mature in size, × 755. FIG. 25. Zoosporangial gall showing exit papilla, × 755. FIG. 26. Zoospores stained with Löffler technique, × 755. FIG. 27. Cleavage of resting spores, × 755. FIG. 28. Resting spore gall, × 575. FIG. 29. Groups of free resting spores, × 575.

TWO NEW SPECIES OF HIRSUTELLA PATOUILLARD

FRAN. E. FISHER

(WITH 4 FIGURES)

During the summer and fall of 1948, epizootics were observed of two economically important arthropods, rust mites [*Phyllocoptruta oleivora* (Ashm.)] and purple scales (*Lepidosaphes beckii* Newm.), on *Citrus* in Florida. The occurrence of a fungus consistently associated with dead first instar nymphs of purple scales was reported by Fisher *et al.* (2), and tentatively identified as a species of *Hirsutella.* Later, a fungus on dead rust mites was also reported (1) as probably being *Hirsutella* sp. Further study has shown that these fungi on dead rust mites and dead first instar nymphs of purple scales are not only separate and distinct species, but also are species which have not previously been described.

Both of these fungi are easily recognized under a dissecting microscope with a magnification of 15 ×. On young purple-scale nymphs, the *Hirsutella* exhibits an extensive growth of mycelium which originates from the ventral surface of the insect's body. Only a few hyphae are present on rust mites; these hyphae typically extend posteriorly or anteriorly from the mite's body, but, occasionally, lateral hyphae are found. The direction of the mycelial growth depends upon the portion of the mite's body from which the hyphae emerge. For instance, if a hypha emerges from the posterior region of a rust mite's body, the typical growth of that hypha will be a direct extension posteriorly from the body.

Since the larger-bodied scales give rise to a fuller mycelium than do the smaller-bodied mites, the variations between the two species were at first considered to be non-specific and to be directly related to the food supply. It has been found, however, that the dissimilarities between these two species are not only in size and extent of mycelium, but also in the shape of the phialides and the spores, and the color of the older mycelia.

Morphologically, neither of these species of *Hirsutella* is entirely typical of the genus. Although the conidiophores are characteristic of *Hirsutella,* the production of synnemata has not been observed on rust mites and only two synnemata have been found on young purple-scale nymphs. Other workers have observed that when growing with an insufficient supply of food often the synnemata are not produced, but, instead, single hyphae. Therefore, it would appear that these species do belong to the genus, *Hirsutella,* but that they grow on such small hosts that they can not, or only infrequently, produce synnemata.

Each of these species exhibits a specificity as to host. The species which attacks first-instar purple scales has not been found on rust mites and *vice versa.* Neither of these species has been observed growing on any other insects which are found on *Citrus.* Whether or not these fungi are responsible for the periodic epizootics of young purple scales and rust mites remains to be proved. However, if they are not parasitic they both appear to be specific indicators of one particular disease of each of the two arthropods.

Hirsutella Besseyi sp. nov. on LEPIDOSAPHES BECKII Newm.

Entomogena. Synnematibus plerumque deficientibus sed raro praesentibus. Mycelio primo albo, deinde cinereo vel brunneo; hyphis erectis vel procumbentibus, 3.3–5.8 μ diam., septatis; conidiophoris a latere vel erectis, rigidis, attenuatis, supra hyalinis infra leve fuliginis, 29.9–74.7 μ longe; phialidibus acuminatis 2.5–4.9 μ × 21.6–66.4 μ, typice in sterigmata attenuatis, 8.3–19.9 μ longe, aliquando 1–3 additicia sterigmata minus 8.3 μ ab phialidis apici gignentibus; conidiis ad apicem, hyalinis, anguste elongatis sed citriformis a capsula gelatinosa visis, 2.5–5.8 μ × 4.1–8.3 μ. Rhizoidibus digitatis.

Hab. in nymphorum primo instar *Lepidosaphes beckii,* in foliis *Citri* spp. prope Lake Alfred, Florida, Amer. Bor. Aug., 1948, Febr., 1949, et Mart., 1949.

Entomogenous. Synnemata infrequently produced. Mycelium white, later gray to brown; hyphae erect or decumbent, 3.3–5.8 μ in diameter, septate; conidiophores lateral or erect, rigid, tapering, hyaline above, pale fuliginous below, 29.9–74.7 μ in length; phialides acuminate, 2.5–4.9 μ × 21.6–66.4 μ, typically attenuated into a sterigma 8.3–19.9 μ in length, occasionally 1–3 additional sterigmata developing within 8.3 μ of phialidal apex; conidia apical, hyaline, narrowly elongate but appearing lemon-shaped due to a gelatinous capsule, 2.5–5.8 μ × 4.1–8.3 μ. Rhizoids digitate.

On first instar nymphs of *Lepidosaphes beckii* Newm., on leaves of *Citrus* spp., vicinity of Lake Alfred, Florida. August, 1948, February and March, 1949.

It has been observed by the author that occasionally large numbers of first-instar purple scales, which are typically white in color, become yellow and a high mortality follows. A species of *Hirsutella* has been found to be consistently associated with nymphs exhibiting the abnormal yellow color.

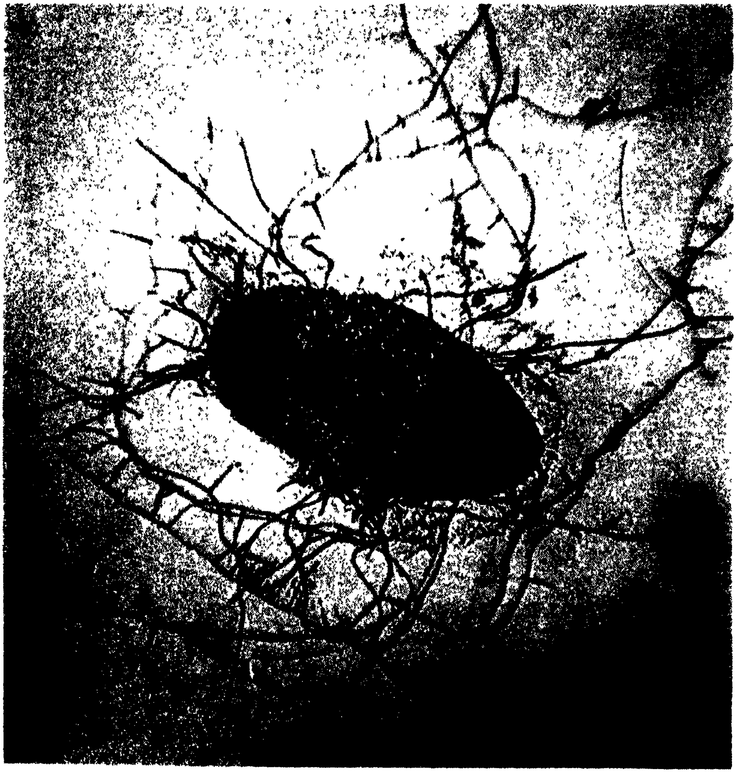


FIG. 1. *Hirsutella Besseyi.*

The mycelium was well developed over the dorsal surface of the host, and was attached to the leaf surface by digitate rhizoids. With a hand lens the fungus appeared translucent to silvery white in color. Older specimens appeared gray to brown.

In early stages the mycelium was fine, hyaline, and septate, later developing thicker cell walls and becoming pale fuliginous in color. Hyphae occurred singly, averaging 4.0 μ in diameter, ranging from 3.3 μ to 5.8 μ. Closely associated parallel hyphae have not been frequently observed. Typically, synnemata were not produced, but in two instances have been found. Figure 1 shows a first-instar nymph of purple scale with a well-developed mycelium bearing conidiophores and conidia.

The fungus fruited abundantly. The conidiophores were lateral or erect and produced regularly on the mycelium. The phialides were acuminate in shape and attenuated into a long sterigma. Occasionally one to three additional sterigmata occurred within 8.3 μ of the apex of the phialide. Phialides bearing more than one sterigma were more commonly observed in older specimens than in young specimens. Each sterigma bore a single, narrowly elongated spore which was surrounded by a gelatinous matrix, or capsule, thereby giving the spore the appearance of being lemon-shaped. Figure 2 shows two conidiophores each of which is attenuated into one sterigma bearing a conidium.

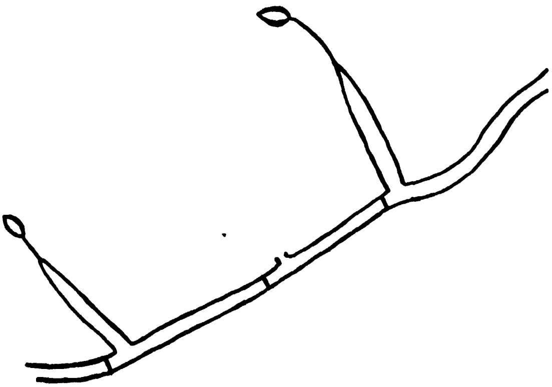
In naming this species, it is the desire of the author to honor a great mycologist, teacher, and friend, Ernst A. Bessey.

Hirsutella Thompsonii sp. nov. on PHYLLOCOPTRUTA OLEIVORA (Ashm.)

Entomogena. Synnematibus deficientibus. Mycelio primo albo deinde griseo; hyphis procumbentibus, 1.7–3.3 μ in diam., breve septatis; conidiophoris a latere vel erectis, rigidis, hyalinis, 10.8–16.7 μ longe; phialidibus conicis ad strobiloidibus, 3.3–4.9 × 5.4–9.9 μ, typice in dua sterigmata attenuatis, 1.7–6.7 μ, saepe uno sterigmato vel aliquando tribus sterigmatis apice gignentibus; conidiis apicibus, hyalinis, ovoidis vel rotundis, citriformis a capsula gelatinosa visis, 2.1–3.3 × 2.1–3.3 μ. Rhizoidibus digitatis.

Hab. in *Phyllocoptruta oleivora* (Ashm.) in foliis *Citri* spp. prope Lake Alfred, Florida, Amer. Bor. Aug., 1948, Febr., 1949, et Mart., 1949.

Entomogenous. Synnemata not produced. Mycelium white, later grayish; hyphae decumbent, 1.7–3.3 μ in diameter, short-septate; conidiophores lateral or erect, rigid, hyaline, 10.8–16.7 μ in length; phialides conoid to strobiloid, 3.3–4.9 × 5.4–9.9 μ, typically attenuated into two sterigmata, 1.7–6.7 μ, but frequently only one sterigma or occasionally 3 sterigmata develop at apex;



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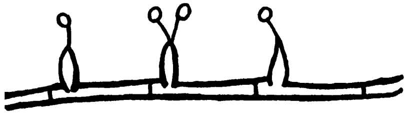

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FIG. 2. Mycelium, phialides, and spores of *Hirsutella Besseyi.* 645 ×.
FIG. 4. Mycelium, phialides, and spores of *Hirsutella Thompsonii.* 645 ×.

conidia apical, hyaline, oval to round, may appear lemon-shaped due to a gelatinous capsule, 2.1–3.3 × 2.1–3.3 μ. Rhizoids digitate.

On *Phyllocoptruta oleivora* (Ashm.) on leaves of *Citrus* spp., vicinity of Lake Alfred, Florida. August, 1948, February and March, 1949.

Type specimens of *Hirsutella Besseyii* and *H. Thompsonii* have been deposited in the Herbarium of the University of Florida, Gainesville, Florida, the Farlow Herbarium, at Harvard University, Cambridge, Massachusetts, and the Herbarium of the Commonwealth Mycological Institute, Kew, Surrey, England.

During October, 1948, an epizootic of rust mites occurred in an unsprayed control plot at Lucerne Park, Florida. Large numbers of the living mites exhibited a dark yellow to tan color rather than the light lemon-yellow color which is typical of healthy rust mites. The characteristics of these abnormal mites correspond to the description by Speare and Yothers (3). Following the occurrence of these large numbers of off-colored mites, a high mortality resulted in the rust mite population. In association with the death of the mites a fungus identified (1) as a species of *Hirsutella* was consistently found to be present on the mites.

Mites which had been dead for a short time and which exhibited a dark yellow to light brown color were mounted for examination.

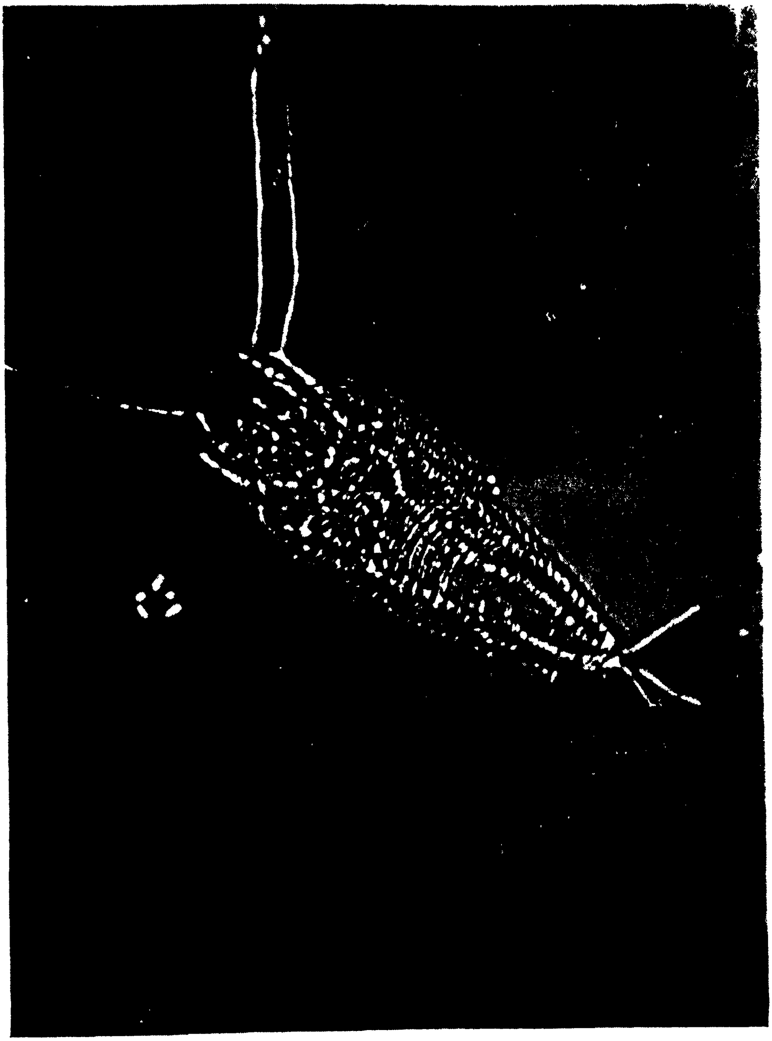

FIG. 3. *Hirsutella Thompsonii.*

Some of these mites contained one or more strands of a fungus mycelium growing longitudinally through the body. On mites which had been dead for a longer period of time, hyphae were growing apparently from the interior through both the anterior and posterior portions of the body. In some specimens lateral emergence of the hyphae occurred. The anterior and posterior emergence of 1–4 hyphae was typical. Under a hand lens, the fungus was silvery-white in color.

During all stages of growth the mycelium was fine and hyaline. The mature hyphae were septate. Hyphae averaged 2.4 μ in diameter, but ranged from 1.7 μ to 3.3 μ. All of the hyphae occurred singly, did not adhere laterally in synnemata, and were attached to the leaves by digitate rhizoids. Figure 3 shows a rust mite with 3 young hyphae growing from both the anterior and the posterior regions. The three hyphae growing from the anterior portion of the mite's body emerged through three of the mite's legs. Several hyphae can be seen extending longitudinally through the body of the mite.

The fungus fruited rather abundantly. Conidiophores were lateral or erect and were produced regularly on the mycelium. Phialides were conoid to strobiloid and attenuated into one or more sterigmata. Although one sterigma was frequently produced by each phialide, the phialides seemed to be typically terminated by two sterigmata, and occasionally three sterigmata were observed on one phialide. Each sterigma bore one oval or round spore surrounded by a gelatinous matrix, or capsule, which in some cases gave the spore a lemon-shaped appearance. Figure 4 shows mycelium bearing three phialides one of which bears two sterigmata.

This species is named to honor a colleague, W. L. Thompson, who has been instrumental in developing the spraying schedule for *Citrus* in Florida, and who provided the author with the first specimens.

ACKNOWLEDGMENTS

The author is indebted to Mr. Erdman West, Botanist and Mycologist, Florida Agricultural Experiment Station, Gainesville, Florida, for the preparation of the Latin diagnoses; to Dr. Ernst A. Bessey, Professor of Botany, retired, Michigan State College,

East Lansing, Michigan, for assistance in the identification of the species herein identified; and to Dr. L. Carl Knorr, Histologist, Citrus Experiment Station, Lake Alfred, Florida, for helpful suggestions in the preparation of the manuscript.

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A MEDIUM FOR THE GROWTH AND MAINTENANCE OF THE YEAST-LIKE PHASE OF HISTOPLASMA CAPSULATUM

E. H. TITSWORTH AND E. GRUNBERG *

(WITH 1 FIGURE)

The production and maintenance of the yeast-like phase of *Histoplasma capsulatum* is known to be difficult. Numerous media have been recommended such as blood agar (Conant, **1941**) and Francis' cystine glucose agar (Campbell, **1947**). These solid agar modifications have, however, a disadvantage in that transfers must be made at short intervals and the yeast-like phase is not obtained consistently.

During recent experiments, in an attempt to obtain the yeast-like phase of *H. capsulatum*, the egg-yolk-potato medium commonly used for the growth of *Mycobacterium tuberculosis* was found to be of value. Twenty-three strains of *H. capsulatum* which were obtained from Vanderbilt University were found to develop a yeast-like phase on this medium within one to two transfers if grown at 37° C. These cultures have maintained their yeast-like phase on repeated subculture for a minimum period of thirty days.

The formula and preparation of the egg-yolk-potato medium (Sasano and Medlar, **1943**) is as follows:

A. Potato base

Potato—peeled and finely ground	200	gm.
Glycerol	60	ml.
Citric acid	0.2	gm.
Bacto-hemoglobin	5	gm.
H_2O	1000	ml.

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* From the Chemotherapy Laboratories, Hoffmann-La Roche Inc., Nutley, N. J.

Autoclave at 15 lbs. pressure for 30 minutes. Filter through a double layer of gauze. Flask in 500 ml. quantities and autoclave at 15 lbs. pressure for 30 minutes.

B. Egg-yolk mixture

Clean eggs, soak in 80 per cent alcohol for 2 hours. Decant alcohol and dry eggs by flaming. Separate whites from yolks into a sterile graduate. A proportion of one whole egg to eleven egg yolks is used. Filter egg plus egg-yolk through a double layer of sterile gauze.

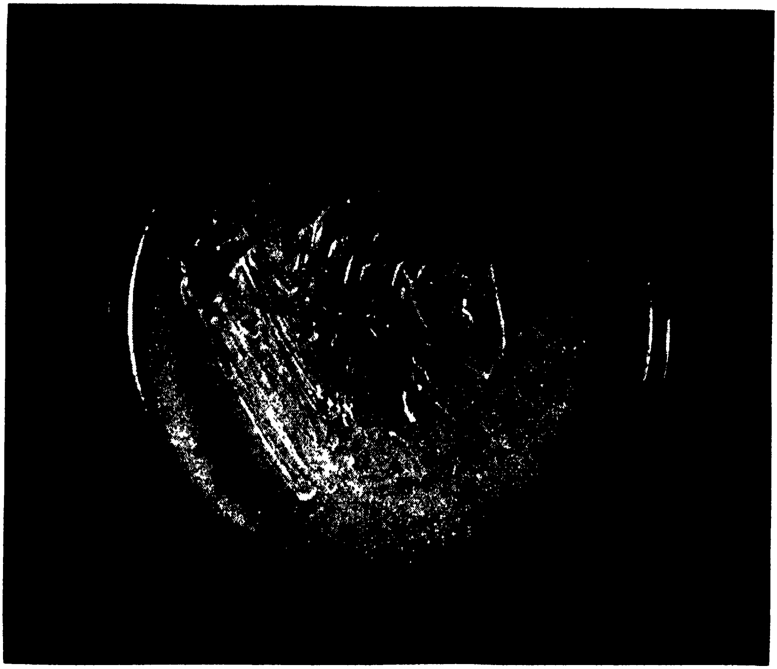

FIG. 1. Yeast-like growth of *Histoplasma capsulatum.*

Mix 500 ml. of the sterile egg-yolk filtrate with 500 ml. of sterile potato-base. Add 10 ml. of sterile 10 per cent congo red. Dispense the mixture into sterile test tubes or Petri dishes as desired and inspissate in free-flowing steam in the autoclave with no pressure for one hour. Incubate for sterility.

All test tubes or Petri dishes should be kept well sealed with paraffin or parafilm to preserve moisture.

An example of the type of yeast-like growth obtained on first or second transfer from the mycelial phase is depicted in figure 1.

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NO BRACHYMEIOSIS IN PYRONEMA CONFLUENS

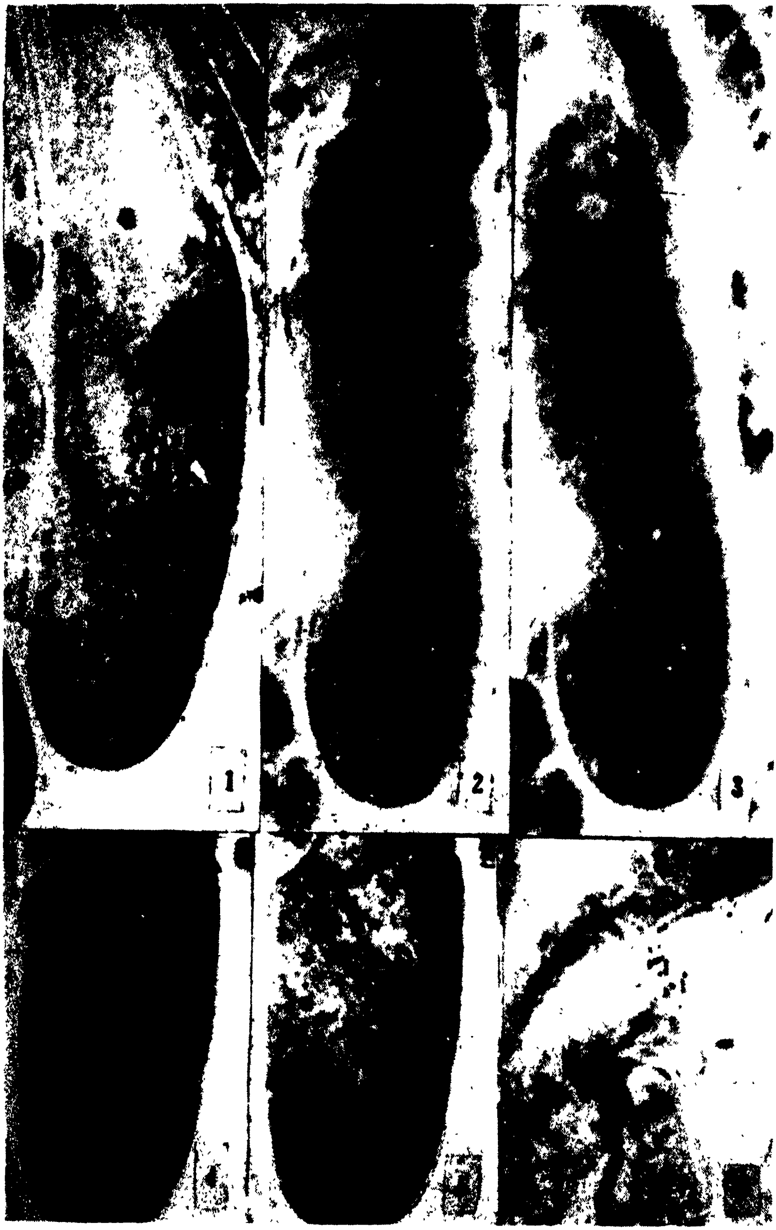
HILDE E. HIRSCH

(WITH 6 FIGURES)

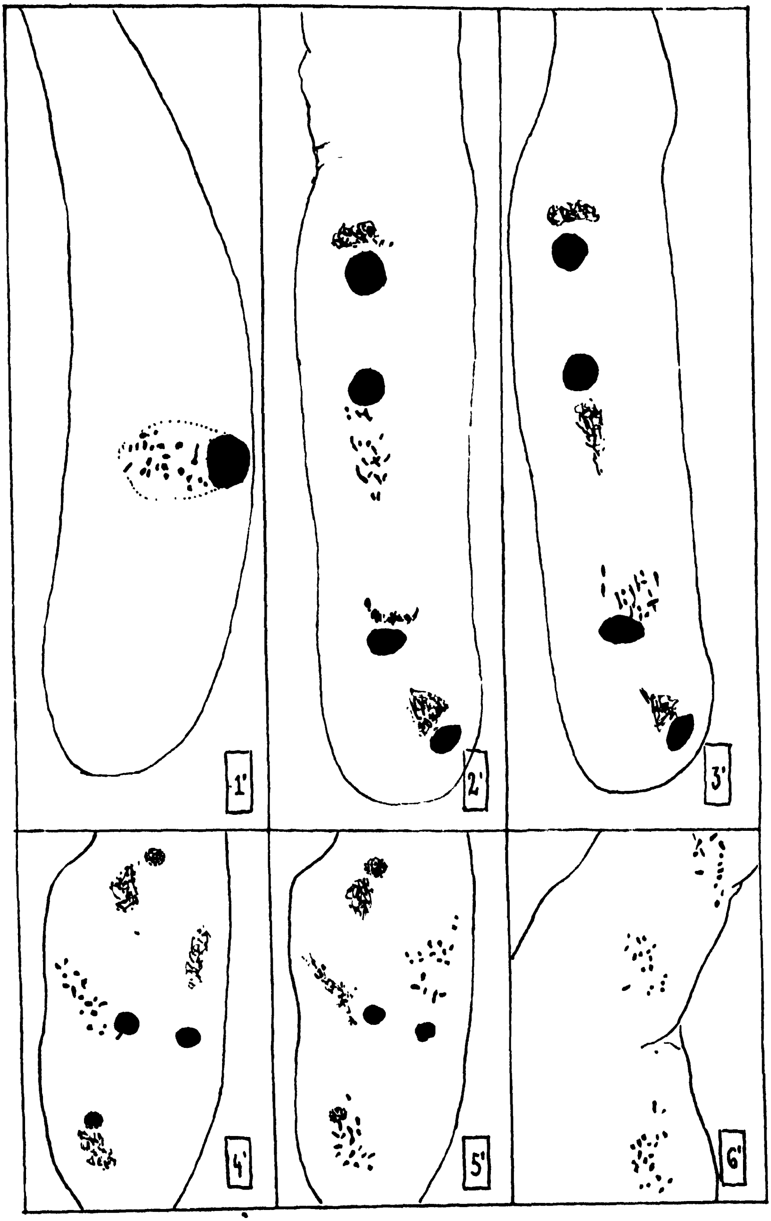
The question whether two reduction divisions occur in the life cycle of at least some ascomycetes is one which was argued vehemently for over 30 years. Swingle (**1934**) has given an excellent summary of the numerous reports supporting or denying the occurrence of two nuclear fusions (one in the ascogonium and one in the ascus), and their necessary consequence, double chromosome reduction or "brachymeiosis." The organism which has played a central role in these investigations and discussions is the well known *Pyronema confluens* Tul. [*P. omphalodes* (Bull.) Fuckel]. More recently, the genetical and cytological work done on a number of ascomycetes has served to strengthen the view that brachymeiosis does not occur. The present brief note is intended to present some photomicrographs of preparations obtained with the acetocarmine smear technique which, in the opinion of the writer, show that in the classical *P. confluens* the claims of two successive reductions in chromosome number are erroneous.

Materials and methods: The strain of *P. confluens* was obtained through the courtesy of Dr. H. N. Hansen. It is the common form and not identical with Brown's var. *inigneum* which is able to grow on unheated substrates and is apogamous (Brown, **1915**). The fungus was grown on Hansen and Snyder's medium (**1947**), consisting of plain agar mixed with pieces of pea straw which have been sterilized by propylene oxide. In the diffuse light outside a north window, it produced apothecia readily and in large quantities. They were fixed in a solution consisting of 1 part glacial acetic acid saturated with iron acetate (Cave and Rattenbury, unpublished), 2 parts absolute alcohol and 3 parts ether, and stained with iron-acetocarmine.

Description of photomicrographs: Figure 1 shows an early anaphase of the first meiotic division in the ascus. 24 Chromosomes



FIGS. 1–6. Division figures in *Pyronema*.



FIGS. 1'–6'. Drawings of figs. 1-6.

are visible, 12 (bivalents) going to each pole. Figures 2 and 3 show the anaphase of the third division in two of the four visible nuclei in one ascus. Similarly, in another ascus, figure 4 shows an early anaphase of the third division in one nucleus, and figure 5 in two other nuclei. Figure 6, finally, shows three third division nuclei in still another ascus. All the nuclei seen in figures 2–5 contain more than 12 chromosomes (although not all 24 can be counted in any one photograph), indicating that more than 6 will pass to each pole.

Discussion: According to Gwynne-Vaughan and Williamson (**1931**), the number of chromosomes in the germinating spore, the cells of the apothecial sheath, paraphyses, etc., is 6 (the haploid number); in the ascogenous hyphae it is 12, and in the definitive ascus nucleus there are 12 gemini (4n). In the third division in the ascus, according to these authors, the number is again reduced to 6. Our observations, on the contrary, confirm the reports of Claussen (**1912**) and of Moreau and Moreau (**1931**), which state that 12 chromosomes go to each pole in the third division, the haploid number being 12. The writer has not been able to count chromosomes in either the somatic cells or the ascogenous hyphae, but in the conjugate division in the crozier, approximately 12 chromosomes were seen.

No attempt was made to observe the behavior of the male and female nuclei in the ascogonium after fertilization, though the movement of the antheridial nuclei through the trichogyne was seen. However, if no brachymeiosis occurs in the ascus, as we believe to have shown, no nuclear fusion in the ascogonium would be expected.

The argument remains that different strains of the fungus may differ with regard to their nuclear behavior. Unfortunately, the original strain of Gwynne-Vaughan and Williamson appears to have been lost, but the strain occurring in Britain should also be reexamined with the modern smear technique.[1]

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[1] Olive's re-investigation of *Patella melalome* (Science **110**: 185 (1949)), published after the above note went to press, has recently shown that in that fungus, too, the claim of brachymeiosis was erroneous.

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EXPLANATION OF FIGURES

FIG. 1. First division of the definitive ascus nucleus, early anaphase. 12 chromosomes (bivalents) moving to each pole. FIG. 2. Anaphase of third division, four nuclei are visible. In the third nucleus from the tip the chromosomes can be counted. FIG. 3. The same ascus as in figure 2, focused to show the chromosomes in the second nucleus from the tip. FIG. 4. Another four-nucleate ascus. Chromosomes can be counted in the left middle nucleus. FIG. 5. The same ascus as in figure 4, focused to show the chromosomes in two other nuclei. FIG. 6. Third anaphase in another ascus. Three nuclei visible. All figures about 1750 ×.

THE GENUS GIBELLULA ON SPIDERS IN NORTH AMERICA[1]

E. B. Mains

(WITH 14 FIGURES)

In 1894 the genus *Gibellula* was proposed by Cavara (4) based on *Gibellula pulchra* (*Corethropsis pulchra* Sacc.). Since then a number of species have been described mostly on spiders. In 1932 Petch (18) published a paper concerning the genus, placing most of the names in synonymy and recognizing only two species, *G. aranearum* (Schw.) Syd. and *G. alata* Petch. The latter is described as a new species on spiders from Ceylon. *G. aranearum* is given as occurring on spiders and possibly on insects (locustids and membracids) throughout most of the world. *Gibellula elegans,* which is placed in synonymy with *G. aranearum,* was described by P. Hennings (8) on locusts from Java. It is doubtful that a species on locusts is the same as one on spiders. The identity of *G. phialobasis* on spiders from Java also is doubtful. If the description and illustration given by Penzig and Saccardo (15) are correct it is distinct from *G. aranearum. Gibellula formosana* Sawada (23) has been reported on a moth from Japan but its assignment to the genus *Gibellula* is very doubtful. Kobayasi (10) states that it belongs very near *Isaria japonica.* In 1949, the writer (11) described *G. formicarum* on an ant from Liberia.

For North America, three species have been reported, *Gibellula aranearum, G. arachnophila* and *G. capillaris.* Morgan (13) described *G. capillaris* in 1905 from a collection on an insect at Preston, Ohio. His description does not suggest a *Gibellula.* Through the kindness of G. W. Martin, the type specimen, which is in the Herbarium of the University of Iowa, has been seen. It consists of only small fragments from which it was not possible to reach a con-

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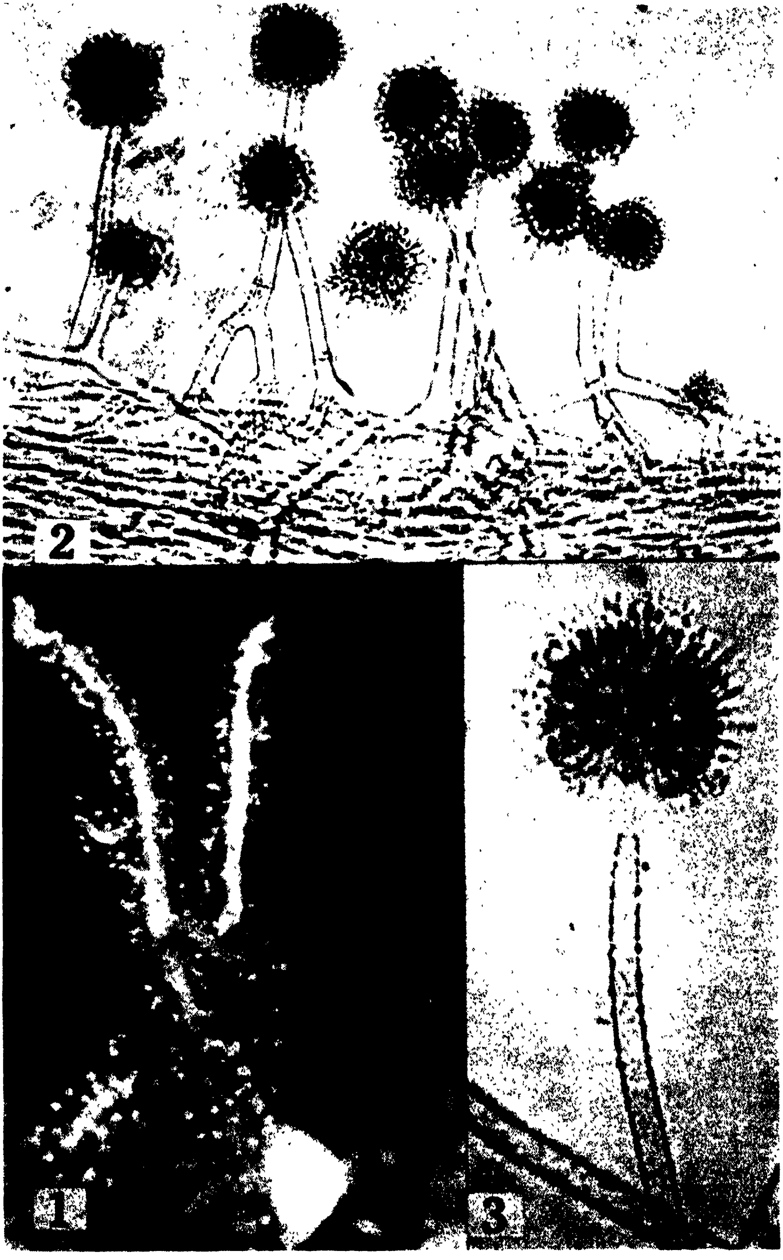
[1] Paper from the Herbarium and the Department of Botany, University of Michigan. The cost of excess illustrations has been paid by the Herbarium of the University of Michigan.



FIGS. 1–3. *Torrubiella arachnophila* var. *pulchra*.

clusion concerning its identity. Both Petch (18) and Vera K. Charles (5) have decided that the species does not belong in *Gibellula*. It is doubtful that its identity can be established. As shown later the name *G. arachnophila* has been misapplied. According to the treatment by Petch (18) one species, *G. aranearum*, is found in North America.

In a study of collections in the Farlow Herbarium,[2] the Herbarium of the New York Botanical Garden, the Mycological Collection of the U. S. Bureau of Plant Industry, and the Herbaria of the University of Michigan and of Cornell University, two species of *Gibellula* have been found to occur on spiders in North America. One is that described by Cavara (4) as *Gibellula pulchra* in establishing the genus. It produces a mat of yellow mycelium covering the host and often attaches the host to the substratum. From the mycelium numerous cylindric synnemata (FIG. 1) arise. These consist of more or less parallel, longitudinal, loosely bound hyphae (FIG. 2) and are covered on the outside by a network of brownish, asperulate hyphae which are loosely attached to the synnemata. The conidiophores develop from the outer loose mycelial covering of the synnemata or from the mycelium covering the host (FIGS. 1, 4). They arise from inverted *T*- or *Y*-shaped cells of the outer mycelial covering, one arm of such cells forming the base of the conidiophore (FIG. 2). The conidiophores vary considerably in length and consist of 2–6 cells; all except the terminal cell have brownish, asperulate walls. The terminal cell of the conidiophore has a hyaline, smooth, or very minutely roughened wall. At its point of attachment it is narrower than the cell to which it is attached (FIG. 3). It enlarges above from a slender neck becoming broadly obovoid or ellipsoid in the upper part. The terminal cell bears a number of broadly obovoid prophialides. From the upper portion of each prophialide 6–8 narrowly clavate phialides are produced. From their apices the conidia develop. The cluster of prophialides and phialides form spherical heads, 40–50 μ in diameter (FIG. 3). The conidia apparently are produced in succession from the apices of

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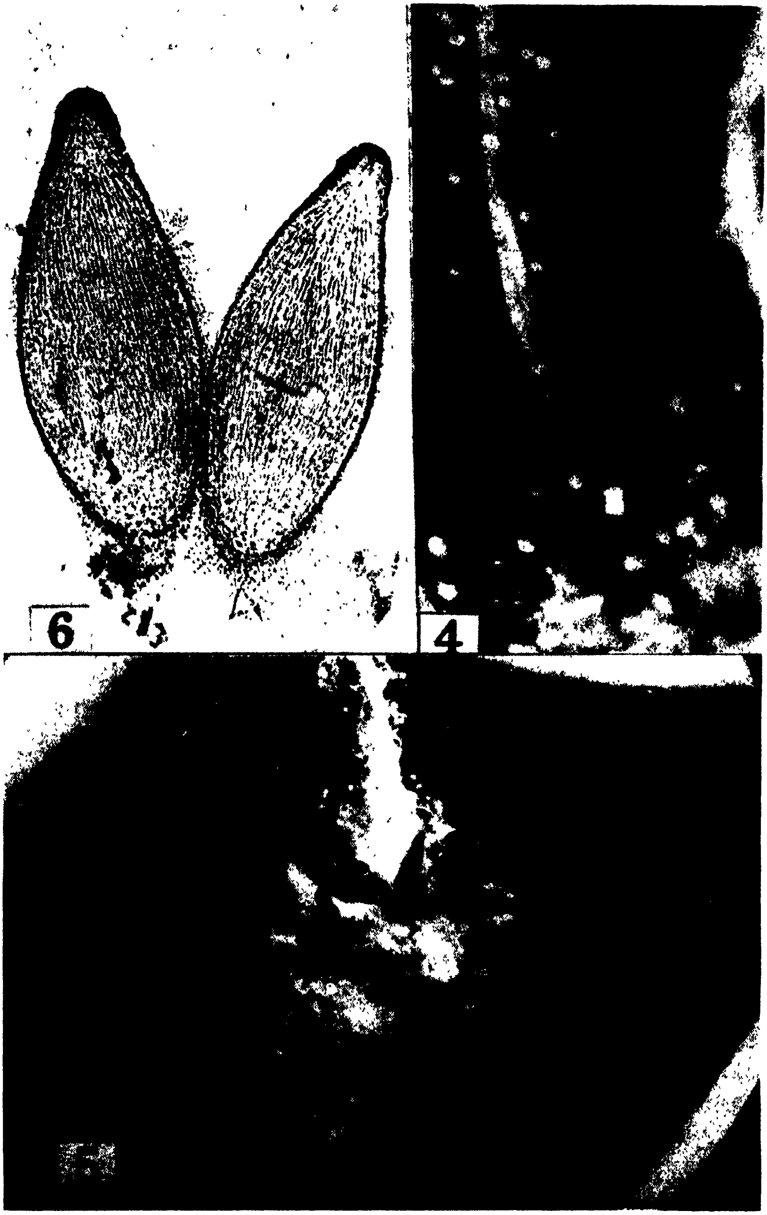
[2] The writer is indebted to W. Lawrance White, Donald P. Rogers, John A. Stevenson and Miss K. E. Kindleberger for the loan of specimens for this study.



FIGS. 4–6. *Torrubiella arachnophila* var. *pulchra*.

the phialides. They usually are found attached singly but in some collections adhere in short chains.

The synnemata with the loose mycelial covering and scattered conidiophores have the appearance of a stilbaceous fungus parasitized by an *Aspergillus* or *Sterigmatocystis* (FIG. 1). Saccardo (22) described it as *Corethropsis pulchra* attacking an *Isaria*. Cavara (4), recognizing its true nature, established the genus *Gibellula* for it and published a good illustration. It also has been well illustrated by Speare (25) under the name *G. suffulta*.

Petch has placed *G. pulchra* in synonymy under the name *G. aranearum* (Schw.) Syd., although Sydow (26) described *G. aranearum* as a new species without any reference to Schweinitz. *Isaria aranearum* was described by Schweinitz (24) in 1822. Fries (7) published the species in his Systema Mycologicum with a description evidently taken from Schweinitz. Unfortunately a specimen no longer exists in the Schweinitz Herbarium at the Academy of Natural Sciences of Philadelphia or in the Michener Herbarium, Mycological Collections, Washington. Therefore it is not possible to determine the microscopic characters of the species and establish with certainty its identity. The description given by Schweinitz is not sufficient to definitely establish the application of the name to one of the species of fungi parasitic on spiders.

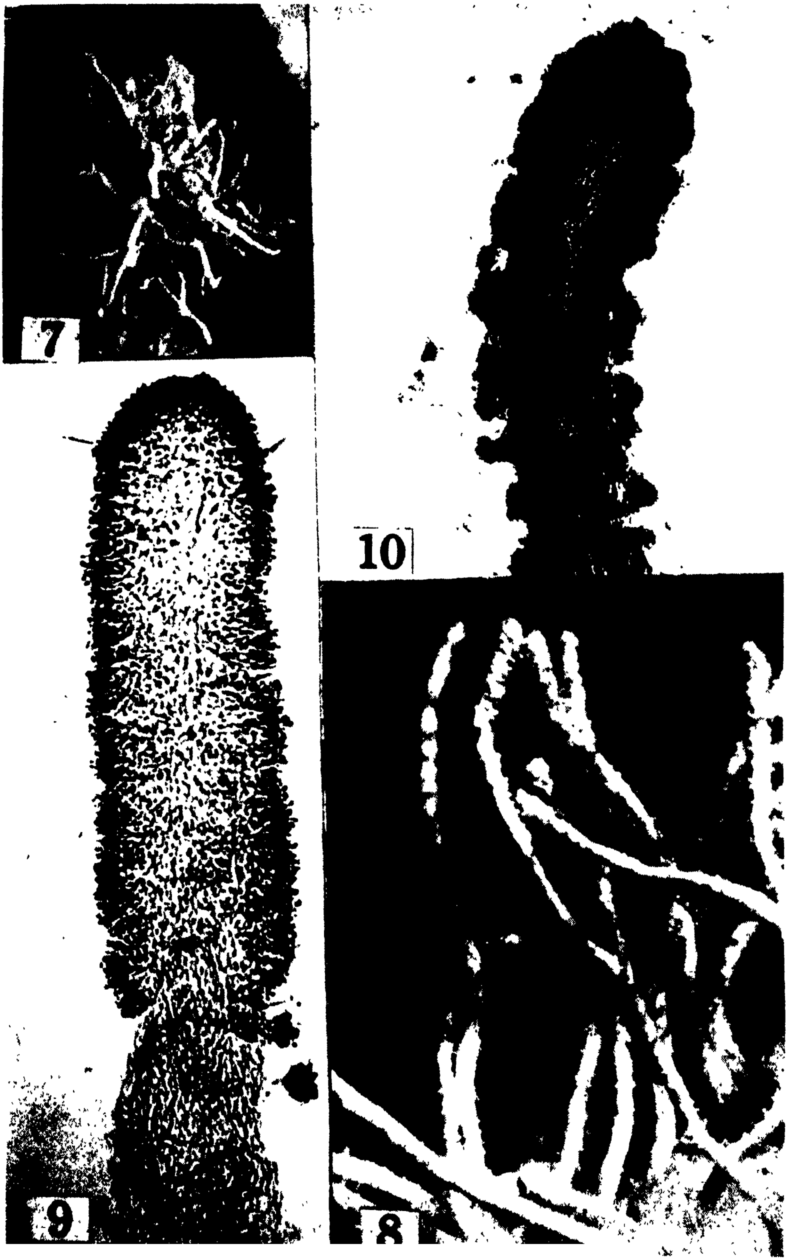
Isaria arachnophila Ditm. on spiders has been reported by several authors who give descriptions and illustrations of a *Gibellula* and Vuillemin (28) transferred it to that genus. Fries (7) in his Systema Mycologicum included other hosts besides spiders for *I. arachnophila*. However, the name and his reference to Ditmar's description and illustrations indicate that *I. arachnophila* should be applied to a fungus on spiders. It seems evident from Ditmar's (6) illustrations that *I. arachnophila* is not a *Gibellula*. It apparently is a *Hymenostilbe* as Petch (17) has pointed out.

Wallroth (29) described a species on spiders under the name *Isaria tartarea*. His description does not indicate *Gibellula pulchra*, which appears to be the first name which can be applied to the species with certainty. The species has since been described and named *Gibellula suffulta* by Speare (25), *G. arachnophila* by

Johnston (**9**), *G. Haygarthii* by Bijl (**1**) and *G. aranearum* by Sydow (**26**).

The other species of *Gibellula* in North America differs from the preceding in several important aspects. It produces conidiophores that are usually much shorter. Often the terminal cell arises directly from the side arm of a cell of the outer mycelial covering of the synnema (FIGS. 11, 12). The length of the conidiophore is usually determined by the length of the side arm of the mycelial cell which may be so short that the terminal cell is practically sessile on the mycelium (FIG. 11) or may be as long as 40 μ (FIG. 12). Occasionally there may be an additional cell between the terminal cell and the mycelium. The broadly obovoid terminal cell of the conidiophore does not have a narrow neck like that of the preceding type but enlarges from a base which is as broad as the cell to which it is attached (FIG. 12). The prophialides are produced from the upper part of the terminal cell and with the phialides form hemispherical to wedge-shaped heads (FIGS. 11, 12), differing thus from the spherical heads of the preceding species. The conidiophores are usually closely crowded, especially over the upper portion of the synnema (FIGS. 9, 10). The almost sessile heads give a tufted appearance to the synnemata which even under low magnifications makes this species easy to distinguish from the preceding (compare FIGS. 7 and 8 with 1 and 4). Petch (**18**) apparently discusses these species as variations of *G. aranearum.* He places most emphasis on length of the conidiophores and states that in some collections of the second type there is a transition from stalked to sessile heads on the same synnema. In such specimens seen by the writer the shapes of the terminal cells and heads of the conidiophores are distinctive. These distinctions justify the recognition of two species.

The second species has been described and illustrated by Oudemans (**14**) and Boudier (**3**) under the name *Isaria arachnophila* Ditm. Vuillemin (**28**) also has discussed and illustrated it under the name *Gibellula arachnophila* (Ditm.) Vuill. giving *Isaria arachnophila* Ditm. as a synonym. Petch (**18**) also concluded that Wallroth (**30**) described this species under the name *I. arachnophila* Ditm., "tegmen tartareum" referring to the tufted condition of the



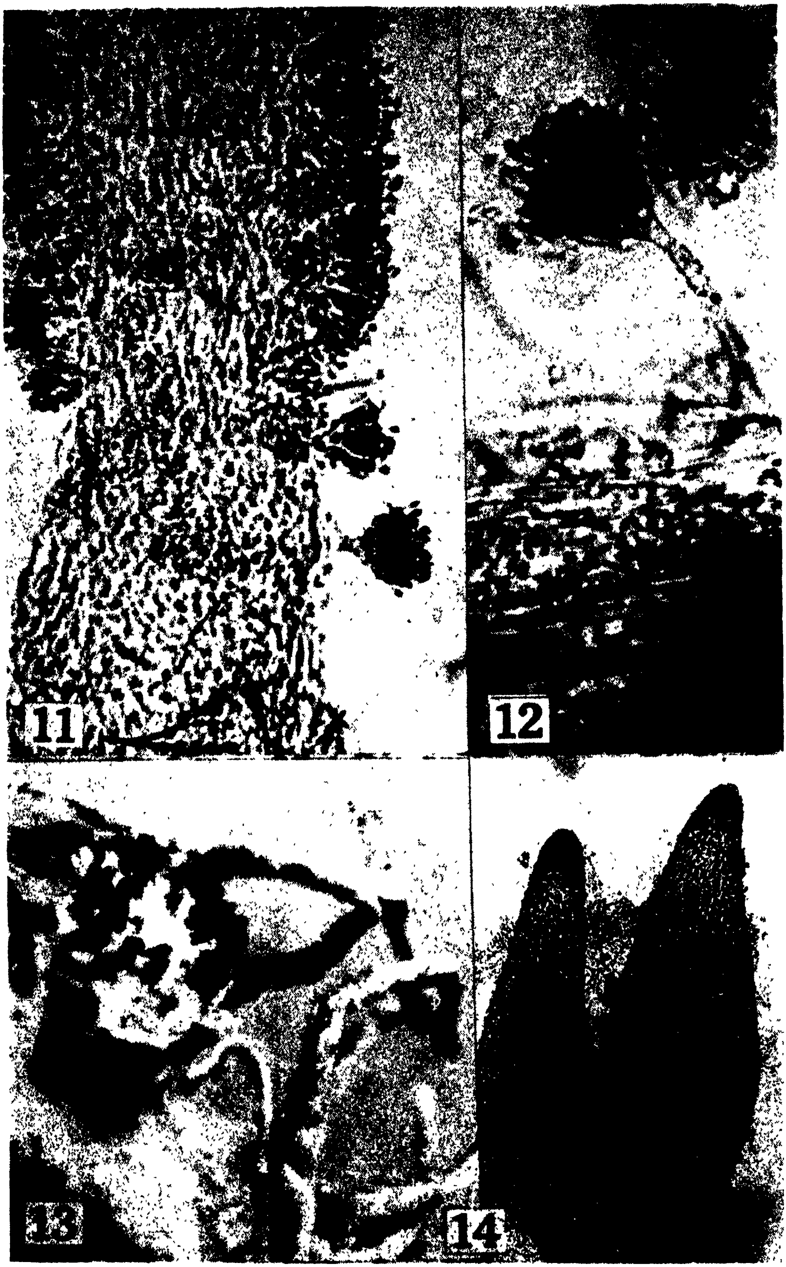
FIGS. 7–10. *Torrubiella arachnophila* var. *pleiopus.*

synnemata. As has already been stated *I. arachnophila* Ditm. is doubtless a *Hymenostilbe* and the name therefore has been misapplied to a *Gibellula.*

In 1920 Maublanc (12) proposed two forms of *G. arachnophila,* forma *macropus* Vuill. and forma *leiopus* Vuill. Although they were distinguished mostly on the lengths of the conidiophores the illustrations of Maublanc and Vuillemin (28) indicate without much question that forma *macropus* is *G. pulchra* and forma *leiopus* is the second species under discussion. As already stated these are separated by other important differences. Therefore the name **Gibellula leiopus** (Vuill.) comb. nov. is proposed for the second species.

Among the North American collections a few specimens of both *G. pulchra* and *G. leiopus* bear perithecia. In the perithecial stage they show no important differences. The perithecia develop in the mycelium covering the host and are at first embedded except for their apices. The shrinkage of the mycelium in herbarium specimens leaves the perithecia only partly embedded but usually with a thin covering of hyphae on the lower portions (FIGS. 5, 6, 13, 14). The asci are long and narrow, containing eight filiform multiseptate ascospores. The perithecial stages of these fungi place them in the genus *Torrubiella.*

Under the name *Cordyceps arachnophila,* Johnston (9) in 1915 described perithecia associated with synnemata of a *Gibellula* on spiders from Cuba. His description is that of a *Torrubiella.* Johnston implies that the species had previously been described since he makes the following statement. "The perfect stage of a Gibellula has at least in one case been described as a Cordyceps. What appears to be this species has been studied by the writer in Cuba." He does not give a reference to the place of publication or the authority for the species. Apparently the only previous use of the name was by Thaxter (27) in 1914. In a paper concerning the genus *Aschersonia* he states that the perithecial stages of some species of *Aschersonia* "might at first be mistaken for the common *Cordyceps* (*Torrubiella*) *arachnophila,* which is often found on leaves with or without its imperfect or *Isaria* (*Gibellula*) condition." A description is not given and the name therefore was not validly



FIGS. 11–14. *Torrubiella arachnophila* var. *pleiopus*.

published. As far as the writer has been able to determine Johnston's publication of *Cordyceps arachnophila* is the first description of perithecia associated with a *Gibellula* on spiders. Since the fungus is a *Torrubiella* the combination *Torubiella arachnophila* is proposed. Johnston describes the conidial stage under the name *Gibellula* (*Isaria*) *arachnophila* without a reference to Ditmar or to other authorities. His description indicates that it is *Gibellula pulchra.* All the collections cited are from Puerto Rico. It is not certain whether the conidial stage accompanying the perithecia of the Cuban material is the same.

Petch (**18**) in 1932 reported perithecia of a *Torrubiella* occurring in four collections having conidiophores of "*Gibellula aranearum*" and has described the perithecial stage under the name *Torrubiella gibellulae* Petch. In these collections synnemata were lacking, the conidiophores occurring on the mycelium with the perithecia. Petch does not describe the conidiophores which accompany the perithecia. *Torrubiella gibellulae* appears to be the same as *T. arachnophila.*

In general the species of *Torrubiella* on spiders show less marked differences in their perithecial stages than in the conidial. The description of *I. flava* Petch on spiders does not indicate important differences from *I. arachnophila.* This conclusion is supported by specimens of the two species determined by Petch in the Farlow Herbarium. However, Petch (**16**) states that the conidial stage of *I. flava* is a *Hirsutella* and therefore it is very distinct in its conidial stage. Several other species of *Torrubiella* on spiders also show much greater differences in their conidial stages than in the perithecial. *Torrubiella aranicida* Boud., the type of the genus, is reported by Boudier (**2**) as having *Isaria cuneispora* Boud. as its conidial stage. Petch (**19**) states that *Cephalosporium aranearum* Petch is the conidial stage of *Torrubiella alba* Petch, *Spicaria longipes* Petch (**20**) that of *T. gonylepticida* (Möller) Petch and *Cylindrophora aranearum* Petch (**21**) that of *T. albolanata* Petch. It is not possible at the present to determine specific limitation and relationships of the species of *Torrubiella.* This must await a restudy of the genus as a whole. It does not seem advisable therefore to propose additional species of *Torrubiella* on spiders on the differences which have been noted. These differences, however, are sufficient to justify the recognition of two varieties of *Torrubiella arachnophila* distinguished by their conidial stages, and consequently *Torrubiella arachnophila* var. *pulchra* with *Gibellula pulchra* Cavara as its conidial stage and *T. arachnophila* var. *leiopus* with *G. leiopus* (Vuill.)Mains as its conidial stage are proposed.

In the following descriptions (FH) indicates that the specimen is in the Farlow Herbarium, Harvard University, (NY) in the Herbarium of the New York Botanical Garden, (W) in the Mycological Collections Bureau of Plant Industry, Washington, (M) in the Herbarium of the University of Michigan and (C) in the Herbarium of the Department of Plant Pathology of Cornell University.

Torrubiella arachnophila (Johnston) comb. nov.

Cordyceps arachnophila Johnston, Bul. Porto Rico Insular Exp. Sta. **10**: 23. 1915.

Torrubiella gibellulae Petch, Ann. Mycol. **30**: 391. 1932.

Perithecia arising from a yellow to yellowish white mat of mycelium covering the host, scattered or crowded in small groups, at first embedded in the mycelial covering except for the apices, becoming more or less superficial, the lower portions covered with a thin layer of hyphae, light yellowish brown to reddish brown above, narrowly ovoid to conoid, 550–1200 × 230–350 μ; asci narrowly cylindric, 450–660 × 4–6 μ, the wall thin, thickened into a cap at the apex, 4–5 μ thick; ascospores filiform, hyaline, nearly as long as the asci, 1.5 μ wide, multiseptate, tardily breaking into one-celled fragments, the cells 4–10 μ long.

Torrubiella arachnophila var. **pulchra** var. nov. (FIGS. 1–6)

Status conidicus *Gibellula pulchra* (Sacc.) Cavara, conidiophoris 2–4 septatis, 150–600 μ longis, cellis terminalis attenuatis deorsum late ellipsoideis vel obovoideis sursum, gerentibus capitula spherica. Perithecia ovoidea, 900–1200 × 300 μ, ascis cylindricis, 600–660 × 5–7 μ, ascosporis filiformibus, multiseptatis. Specimen typicum in araneis, Burbank, Tenn., R. Thaxter, 4122, Aug. 1896, in Herb. Mich.

The distinctive conidial stage of the variety is *Gibellula pulchra* for which the following description is given. All the collections listed have the conidial stage. The one collection with perithecia is noted.

GIBELLULA PULCHRA (Sacc.) Cavara (FIGS. 1–4)

Att. Instit. Bot. Univ. Pavia Ser. II, **3**: 347. 1894.

Corethropsis pulchra Sacc., Michelia **1**: 83. 1877.

Corethropsis australis Speg., An. Soc. Cient. Arg. **13**: 24. 1882.

Gibellula suffulta Speare, Phytopath. **2**: 137. 1912.

Gibellula arachnophila Johnston, Bul. Porto Rico Insular Exp. Sta. **10**: 24. 1915. Not *Gibellula arachnophila* (Ditm.) Vuill. 1911.

Gibellula arachnophila f. *macropus* Vuill. apud Maublanc, Bul. Soc. Mycol. France **36**: 41. 1920.

Gibellula Haygarthii Bijl, Trans. Roy. Soc. S. Africa **10**: 149. 1922.

Gibellula aranearum Syd., Engler's Bot. Jahrb. **62**: 321. 1922.

Synnemata arising from a yellow or yellowish mat of mycelium covering the host, cylindric, yellowish brown to violaceous brown when dried, more violaceous when fresh, sometimes slightly enlarged above, 3–9 mm. long, 100–300 μ thick below, 200–400 μ thick above, consisting of multiseptate somewhat loosely bound longitudinal hyphae; conidiophores arising from the mycelium covering the host or from a network of brownish asperulate hyphae loosely attached to the surface of the synnemata, 150–600 μ long, 7–12 μ wide, the lower 3–5 cells brownish, asperulate, the terminal cell hyaline, smooth, 2–3.5 μ thick below, enlarging from a slender neck into a broadly ellipsoid or obovoid head, 6.4–10 μ wide bearing up to 12 broadly obovoid prophialides measuring 6–12 × 4–6 μ, each prophialide in turn bearing at the apex clusters of 6–8 narrowly clavate phialides measuring 6–10 × 2–3 μ, the prophialides and phialides forming spherical heads 30–42 μ in diameter; conidia fusoid to fusoid-ellipsoid, 2.5–6.4 × 1.5–2.5 μ, produced in succession from the apices of the phialides, occurring singly or in short chains.

On spiders.

Delaware: Noaman's Creek, Brandywine Hd., Aug. 16, 1892, A. Commons (NY).

Maine: York, Aug. 1895, R. Thaxter (FH *6247*), Sept. 1895 (FH *6239*); Millinocket, 8-23-40, Steinmetz (C *29183*).

Massachusetts: Waltham, Sept. 1886, R. Thaxter (FH *6268*); Canton, Aug. 1936, A. M. & D. P. Rogers *2091* (NY).

North Carolina: Cranberry, 1887, R. Thaxter (FH *6253*).

Tennessee: Burbank, Aug. 1896, R. Thaxter, perithecia, type (M and FH

4122); Cades Cove, Great Smoky Mt. Nat. Park, Aug. 20, 1939, D. H. Linder (FH).

Puerto Rico: Rio Piedras, Aug. 31, 1913, J. R. Johnston (FH *6283*); Rio Piedras, Dec. 27, 1913, J. R. Johnston (FH *6282*); San Juan, March 2, 1923, G. N. Walcott (W); Arecibo, Faxon, Mills & Anderson *2940* (W); Barceloneta, Nov. 20, 1934, C. G. Anderson & A. S. Mills (W); Pueblo Viejo, Faxon, Anderson and Mills (W).

The following collections from outside of North America have been seen.

Venezuela: Margarita, June 1948, Blakeslee (FH *754*); Aragua, April 5, 1938, Carlos E. Chardon, H. H. Whetzel, A. S. Müller *2786* (C); Central Lucinda, Yaracuy, Carabobo, Jan. 8, 1938, Carlos E. Chardon *2444* (C); Rancho Grande, Aragua, March 28, 1939, E. Chardon, H. H. Whetzel, A. S. Müller *3235* (C).

British Guiana: C. B. Williams, Aug. 28, 1916 (FH *748*).

Trinidad: 1912–13, R. Thaxter (FH *2572*); Maraval Valley, 1912–13, R. Thaxter (FH *2574*); Port-of-Spain, 1912–13, R. Thaxter (FH *6222B*, *6223*); Maraval Reservoir, Port-of-Spain, Feb. 8, 1913, R. Thaxter (FH *6124*); La Seiva Valley, May 6, 1913, R. Thaxter (FH *2564*); St. Anne Valley, June 1913, R. Thaxter (FH *2571*); Feb. 1917, J. B. Rorer (FH *639, 2566*); Port-of-Spain, J. B. Rorer (FH *2585*).

Chile: Corral, 1905–06, R. Thaxter (FH *6119B*).

Territory Hawaii: Kokee, March 9, 1898, C. L. Shear (W).

New Guinea: Morobe, Nov. 11, 1939, Mrs. Clemens (W).

Torrubiella arachnophila var. **pleiopus** var. nov. (FIGS. 7–14)

Status conidicus, *Gibellula pleiopus* (Vuill.) Mains, conidiophoris 1–2-septatis, brevibus, cellis terminalibus late obovoideis vel obpyriformibus non attenuatis deorsum, gerentibus capitula hemisphericia, cuneiformia vel obconica. Perithecia ovoidea, 550–900 × 230–350 μ, ascis cylindricis, 400–600 × 5–6 μ, ascosporis filiformibus, multiseptatis. Specimen typicum, Newfane Hill, Vermont, Aug. 1940, V. K. Charles in Herb. Mich.

The distinctive conidial stage of the variety is *Gibellula pleiopus* for which the following description is given. All the collections listed have the conidial stage. The few with perithecia are so noted.

GIBELLULA PLEIOPUS (Vuill.) Mains (FIGS. 7–12)

Gibellula arachnophila f. *pleiopus* Vuill. apud Maublanc Bul. Soc. Mycol. France **36**: 42. 1920.

Synnemata arising from a yellow to whitish mat of mycelium covering the host, cylindric, 1.5–8 mm. long, 80–300 μ thick, consisting of multiseptate longitudinal hyphae somewhat loosely bound together, light grayish brown to violaceous brown when dried, often reported as violaceous when fresh, the surface having a tufted appearance; conidiophores very short, often scattered on the lower part of the synnemata, usually crowded on the upper part, especially over the apices, arising from a network of brownish, asperulate hyphae loosely attached to the surface of the synnemata, usually the lower portion of a conidiophore being a lateral arm of a cell of the hyphal net, the terminal cells usually produced directly on the side arms, occasionally having an intermediate cell, the lower portion of the conidiophore brownish and asperulate, the terminal cells hyaline and smooth below as wide as the hypha to which it is attached, enlarging upward, broadly obovoid or obpyriform, 10–18 × 4.5–8 μ, bearing a number of prophialides on the upper portion, the prophialides broadly obovoid to ellipsoid, 7.5–12 × 3–5 μ, bearing up to 4 phialides at their apices, the phialides narrowly clavate to subcylindric, 7.5–12 × 2.5–3.5 μ, the walls thin below, slightly thickened at the apices, the prophialides and phialides together forming wedge-shaped to broadly obconic heads, 30–50 μ across; conidia fusoid to fusoid-ellipsoid, 3–8 × 1–2 μ, produced successively from the apices of the phialides, occurring singly or in short chains.

On spiders.

Maine: Kittery Point, Aug. 1886, R. Thaxter (FH *6260*); Kittery Point, July 1886, R. Thaxter (FH *6270*); Kittery Point, Sept. 1891, R. Thaxter (FH *650*); Kittery Point, 1895, R. Thaxter (FH *6251*); Kittery Point, R. Thaxter (FH *6206*).

Maryland: Newfield, Oct. 10, 1875, Ellis Coll. (NY).

Massachusetts: Woods Hole, Sept. 22, 1881, Trelease (FH); Waltham, Sept. 1886, R. Thaxter (FH *6272*); Canton, July 11, 1936, D. H. Linder (FH); Hamilton, July 18, 1937, D. L. Vickar (FH); Harvard Forest, Petersham, Aug. 19, 1949, C. Alexopoulos and C. T. Rogerson *3186* (M).

New Hampshire: Chocorua, Aug. 1904 (FH); Chocorua, Sept. 1906, W. G. Farlow (FH); Chocorua, Aug. 4, 1907 (FH); Shelbourn, Aug. 8, 1940, V. K. Charles (W).

New York: Coy Glen, Ithaca, Aug. 22, 1935, J. A. Stevenson (W).

North Carolina: Cranberry, 1886–1887, R. Thaxter (FH *6159*); Cranberry, Aug. 1887, R. Thaxter (FH *755*, *6263*); Cranberry, 1887, R. Thaxter (FH *6261A*); Cranberry, Aug. 2, 1896, R. Thaxter, perithecia (FH *6169*).

New Jersey: Newfield, March 29, 1896, Ellis Coll. (NY).

Vermont: Newfane Hill, Aug. 1939, V. K. Charles, perithecia, several collections (M, W); Newfane Hill, Aug. 1940, V. K. Charles, perithecia, type (M, W); Newfane Hill, Aug. 1941, V. K. Charles, several collections (W).

Virginia: Great Falls, June 11, 1919, C. H. Kauffman (M); Hoover Camp, Nov. 1, 1935, J. A. Stevenson (W); Mountain Lake, Sept. 4, 1936, A. E. Jenkins (W); Mountain Lake, Sept. 4, 1936, J. A. Stevenson (W); Limberlost, Sept. 9, 1937, V. K. Charles (W); Sept. 9, 1937, J. A. Stevenson (W).

Wisconsin: Madison, 1882, Trelease (FH); Madison, Nov. 19, 1884, Wm. Trelease (FH *6420*).

Nova Scotia: Brookside, July 13, 1931, L. E. Wehmeyer *1015, 1015A* (M).

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EXPLANATION OF FIGURES

FIGS. 1–6. *Torrubiella arachnophila* var. *pulchra*. FIGS. 1–4, the conidial stage, *Gibellula pulchra*; 5, 6, the perithecial stage. FIG. 1. Synnemata arising from the host showing scattered conidiophores, × 15. FIG. 2. Conidiophores arising from the outer loose mycelium of a synnema, × 280. FIG. 3. Upper portion of a conidiophore showing asperulate wall of a lower cell, narrow neck of the terminal cell and spherical cluster of prophialides, phialides and conidia, × 700. FIG. 4. Showing conidiophores arising from the mycelium as well as from a synnema, × 150. FIG. 5. Perithecia partly embedded in the mycelium covering the host, × 15. FIG. 6. Perithecia, × 80.

FIGS. 7–14. *Torrubiella arachnophila* var. *pleiopus*. FIGS. 7–12, the conidial stage, *Gibellula pleiopus*; 13, 14, the perithecial stage. FIG. 7. Synnemata arising from the host, × 3. FIG. 8. Synnemata showing tufted surface, × 15. FIG. 9. Short synnema showing the short conidiophores in a compact layer, × 100. FIG. 10. Upper portion of a synnema showing tufted conidiophores, × 100. FIG. 11. Showing conidiophores consisting of a wedge-shaped head of prophialides and phialides borne on sessile terminal cells, × 200. FIG. 12. Conidiophore consisting of a short asperulate cell, obovoid terminal cell and wedge-shaped head of prophialides and phialides, × 500. FIG. 13. Perithecia partly embedded in the mycelium covering the host, note associated synnemata, × 15. FIG. 14. Perithecia, × 80.

A NOTE ON NAUCORIA MYOSOTIS

Alexander H. Smith[1]

(WITH 1 FIGURE)

This striking species of our northern regions and bogs was transferred to *Flammula* by Singer (Rev. de Myc. **2**: 241. 1937) and placed in the subgenus Phaeo-Naematoloma of *Flammula*. Favrè has given a detailed account of the fungus (Bull. Soc. Myc. Fr. **52**: 143. 1936). In setting up his subgenus Singer was impressed by the resemblance of the fungi he placed here to the slender species of *Naematoloma* such as *N. udum, N. ericeum,* etc. He made no mention of the characteristic naematolomoid pleurocystidia in his diagnosis of the subgenus. Presumably, the members of this group did not possess them and that, of course, would be a good reason for excluding the species from *Naematoloma*.

In connection with a work which is in progress on *Galerina*, North American collections of *N. Myosotis* were examined to check the possibility of placing the species in that genus. All our collections were found to have the characteristic pleurocystidia of the genus *Naematoloma*. Since Favrè made no mention of such organs in his account of the species, an examination of specimens kindly sent us by Favrè was made. Pleurocystidia of the *Naematoloma* type were found; in fact from our study it was clear that no significant differences between the American and European specimens existed.

Smith (Mycologia **40**: 670. 1948) pointed out previously that if the group of genera *Stropharia, Naematoloma* and *Psilocybe* were to be established on a natural as opposed to an artificial basis, spore color in deposits would have to be given less emphasis than was given it in the Friesian classification, *i.e.*, some species with dark brown spore deposits, but with all the anatomical and morphological features of these genera, would have to be admitted to them.

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[1] **Papers from the Herbarium and the Department of Botany, University of Michigan, Ann Arbor, Michigan.**

Naucoria Myosotis is a good representative of this group and is hereby transferred to *Naematoloma.*

Naematoloma Myosotis (Fr.) comb. nov. (= *Agaricus Myosotis* Fr. Syst. Myc. 1: 290. 1821)

Pileus (10) 15–30 (40) mm. broad, broadly conic to convex, glabrous except occasionally with scattered veil remnants along the margin, edge often appendiculate, viscid to glutinous, evenly "medal bronze" to "citrine" (olivaceous or greener), evenly colored, opaque when young but before fading becoming brownish and striatulate, near olive buff faded; flesh dull greenish, pliant, odor and taste not

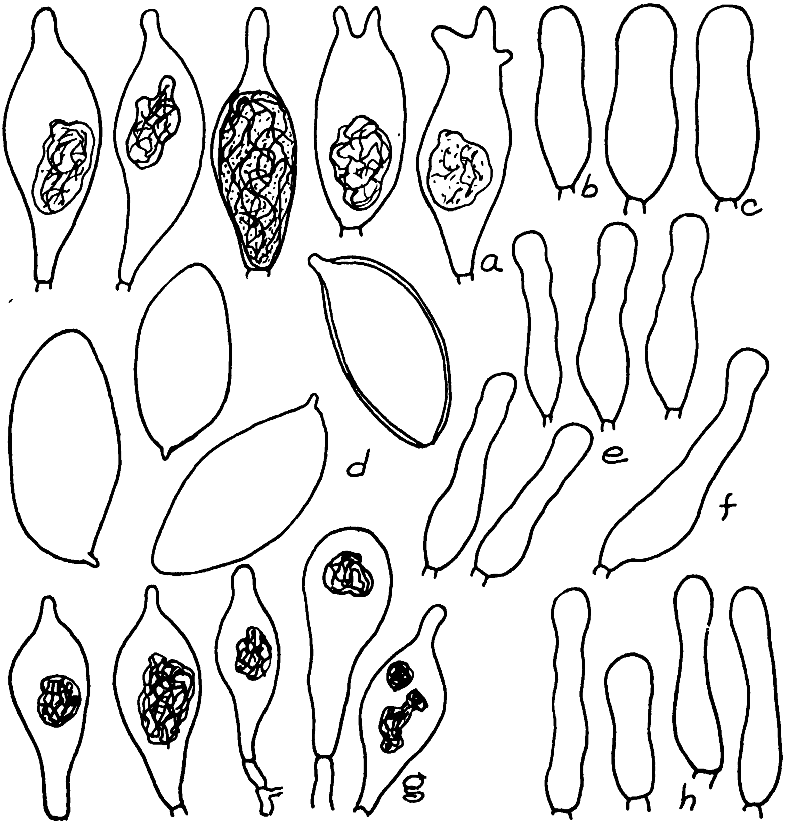

FIG. 1. Spores and cystidia of *N. Myosotis:* a, b, c, d, e, f—from sm–33–988. g and h—from a collection by Favrè.

distinctive; lamellae adnate to shallowly adnexed, equal to slightly ventricose, subdistant, broad, whitish at first, then pale olivaceous and finally brown from the spores, edges minutely white fimbriate and eroded; stipe (6) 10–15 (20) cm. long, 2–5 mm. thick, very rigid, equal, hollow, covered to line formed by the broken veil by white to olivaceous veil fibrils in patches or zones, densely pruinose above.

Spores 14–17 × 7–9 μ, narrowly inequilateral in side view, narrowly ovate in face view, ochraceous tawny in KOH and smooth to very slightly wrinkled (as revived in KOH), the wall relatively thick, apical pore present but apex not truncate; basidia four-spored, projecting when sporulating, hyaline in KOH, 26–34 × 9–11 μ; pleurocystidia present, 35–46 × 10–13 μ, ventricose-mucronate or with several protuberances over upper portion, some ventricose with broadly rounded apices, hyaline and homogeneous in water mounts of fresh material but with a highly refractive amorphous content variously disposed as revived in KOH; cheilocystidia abundant, 32–46 × (6) 8–12 μ, the broadest cells usually with highly refractive content, narrowly ventricose and apices obtuse, thin-walled; gill trama subparallel, olivaceous to yellow in KOH; pileus trama with a well differentiated gelatinous pellicle of hyphae 3–5 μ diam., cells beneath much larger and yellow in KOH (content olivaceous fresh), clamp connections present.

Habit, habitat and distribution: Gregarious on wet humus along edges of bogs, late summer and fall. It is typically northern in its distribution. The description is from Smith 33–988, Huron Mts., Sept. 16, 1933, Mains and Smith. The drawings are from this collection and one by Favrè from the Alps.

NOTES AND BRIEF ARTICLES

ARTICLE 57

CHARLES GARDNER SHAW[1]

Article 57 has been cited so many times in discussions of nomenclatorial problems that most mycologists interested in such matters should be able to quote it *verbatim.* Nevertheless, the first two paragraphs of the article are reprinted once again.

> Art. 57. Among Fungi with a pleomorphic life-cycle the different successive states of the same species (*anamorphoses, status*) can bear only one generic and specific name (binary), that is the earliest which has been given, starting from Fries, *Systema,* or Persoon, *Synopsis,* to the state containing the form which it has been agreed to call the perfect form, provided that the name is otherwise in conformity with the Rules. The perfect state is that which ends in the ascus stage in the *Ascomycetes,* in the basidium in the *Basidiomycetes,* in the teleutospore or its equivalent in the *Uredinales,* and in the spore in the *Ustilaginales.*
>
> Generic and specific names given to other states have only a temporary value. They cannot replace a generic name already existing and applying to one or more species, any one of which contains the "perfect" form (2, 3).

It has been stated recently (in a discussion of *Milesina* vs. *Milesia*) that a name "given" with a description of an imperfect state of a fungus is also "given" to the perfect state of the same fungus, even though no mention of the perfect state is made, if reexamination proves that the perfect state is actually present in the type material (4, p. 250–251). The argument is carried further in a discussion concerning *Albugo* and *Cystopus* (5, p. 432–433).

> The applicability of *Albugo* and of *Cystopus* Lév. to the perfect state depends not on whether the authors who published them recognized the presence of oospores, but on whether oospores were present in their specimens. To argue otherwise would be to discard, as names applied to the imperfect state, all names applied to Basidiomycetes without recognition of

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> he presence of basidia—i.e., most names published before 1837, and many ater ones. It seems reasonable to suppose that at least a part of the material hat Persoon (or Gray) and Léveille had in hand contained oospores, even hough the authors did not describe them. It is therefore submitted that heir names are not rendered "of only temporary value" by Article 57 (5).

I cannot agree that a name has been "given" to a state which the author of that name did not mention in any way in his description. Nor do I believe that this doctrine necessitates abandoning most early names for Ascomycetes and Basidiomycetes.

A name published without a description is a *Nomen nudum* (Art. 37). A name published on printed labels attached to herbarium packets and distributed with the specimens does not constitute valid publication of that name (Art. 37), unless there is also a description (or reference to a previously published description), in spite of the fact that the specimen contained in the packet may be the best material that any past, present, or future mycologist might wish. The name printed and published on the packet in which that specimen is contained is a *Nomen nudum,* in spite of the fact that the name has a type. In other words, article 37 takes precedence over article 18. Stated another way the binomial on the packet is not "in conformity with (all) the Rules" (Art. 57).

It is my contention that article 57 as now worded makes *Nomina provisiora* of all binomials "given" to anything other than the perfect states of pleomorphic fungi. In this connection the following excerpt of article 57 is important: "The earliest (name) . . . given . . . to the state containing . . . the perfect form, *provided that the name is otherwise in conformity with the Rules."* I interpret this to mean that article 37 (as well as all the rest of the Rules) must be complied with. Therefore, the name "given" to the perfect state must be a validly published name *for that state, i.e.,* it must be "accompanied by a description . . . or by a reference to a previously and effectively published description" of that state (Art. 37). (The wording of article 57 would certainly be improved if "given . . . to" were changed to "validly published for.")

If descriptions of the perfect state are necessary to validate names among the Oomycetes and Uredinales, then they are also required for the perfect states of other groups (*e.g.,* Ascomycetes and Basidiomycetes). Rogers, then, sees a possibility that many of the earlier generic names applied to Basidiomycetes may be invalid (4, 5). This may be the main reason for his arguments against the necessity of a description of the perfect state.

Any attempt to circumvent the requirement of a published description for the stage on which the nomenclature of an organism is based must be resisted.

The size of any fungus collection is limited; the number of cotypes into which the original collection can be divided is usually small and consequently distribution of the type material is limited. Often the original collection is sufficient for but one specimen which is deposited in a single herbarium. Neglect, the ravages of time, repeated examination of the type by an ever increasing number of mycologists, or other acts of man could destroy all of the type material of a given species.

The printed word is much more permanent. Once a publication is distributed, neither a single catastrophe nor even several simultaneous catastrophes are likely to destroy all copies of a given publication. Publications are man made. When the reservoir of a publication becomes depleted, it can be reproduced (as has been done with Saccardo's "Sylloge" and as has been proposed for certain works of Persoon, Fries, Sydow, etc.). Therefore publications have permanency.

The material contained in a type collection is not man made and cannot be reproduced by man or otherwise exactly duplicated when it is lost or destroyed. It is an unassailable fact that the last sentence of article 18 now applies to many species; it is my contention that time only will determine the date when it must be applied to all but the most recently discovered species. "Where permanent preservation of a specimen or preparation is impossible, the application of the name of a species . . . is determined by means of the original description or figure" (Art. 18).

A description then has greater permanency, not to mention greater accessibility, than the original specimen. Furthermore a description of the perfect state must be "given" (*i.e.,* validly published) if article 57 and the rest of the Rules are complied with.

The law on this point is *not* contained in article 18 (4, p. 251), but rather in the Rules as a whole.

Even so article 57 does not necessitate abandoning a great many of the earlier names given to Basidiomycetes and Ascomycetes. Let us determine what the perfect state is. Rogers has gone into this thoroughly and concludes "the one meaning of 'state' that makes sense with the whole text of the Rule and with all the examples is 'fructification' " (4, p. 246). With this I agree. The second sentence of article 57 then reads: "The perfect *fructification* is that which *ends* in the ascus state in the *Ascomycetes,* in the basidium in the *Basidiomycetes,* in the teleutospore or its equivalent in the *Uredinales,* and in the spore in the *Ustilaginales.*" Note it is not stated that the perfect fructification *is* the ascus, the basidium, the teleutospore, or the spore respectively, but merely "that which ends" in these structures, *i.e.,* the ascocarp, the basidiocarp, the telium, and the smut sorus.

Since previous discussions concerning this point have used the Oomycetes, the Uredinales, and the higher Basidiomycetes as examples, the same examples are used here. What is the perfect fructification (state) in the Oomycetes? It is the oogonium and the oospore(s) contained therein (and possibly also the antheridium). There are no surrounding sterile fungous structures making up a part of the fructification. The oogonia (containing oospores) are scattered throughout the cortical tissues of the host; there may be a great many or a very few in a given specimen, but there is no organization into a more complex structure. Thus, the perfect fructification in the Oomycetes is the oogonium and its contents, the oospore (or oospores). In order to comply with article 57 and the rest of the Rules, the oogonium and its contents must be described. I have indicated elsewhere that article 57 as now worded does apply to the Phycomycetes (6).

In the Uredinales the perfect fructification is the telium or teleutosorus ("that which ends . . . in the teleutospore or its equivalent"). The telium is essentially a mass of teliospores, although in some species (*e.g., Puccinia allii*) paraphyses are present, and in some microcyclic rusts (*e.g., Endophyllum sempervivi*) a peridium is present (1). The telium (the perfect fructification) then, whatever its shape, structure, and component parts, is the structure which must be described if article 57 is complied with. (Rogers (4, p. 241) has pointed out that in the authoritative French text of the Rules (3) *Uredo* is considered an imperfect state.) It seems doubtful that a telium can be described adequately without mentioning its primary, and frequently only, morphologically significant component, the individual teliospores.

In the Homobasidiomycetes the perfect fructification (state) is a much more complex structure than that of either the Oomycetes or the Uredinales. The basidiocarp is usually composed of several distinct tissues and many different structures of which the basidium is but one. Furthermore, here, to a much greater extent than in the two previously cited groups, the perfect fructification exhibits many macroscopic characters which can be described and used as taxonomic criteria. The basidiocarp (the perfect fructification) then is the structure which article 57 requires be described; article 57 does not require that the basidium itself be described. The situation in regard to the Ascomycetes is similar to that in the Basidiomycetes; the ascocarp must be described, not necessarily the ascus.

The comparable perfect fructifications in the groups discussed are the oogonium and its component parts (few in number), the ascocarp and its component parts, the telium and its components (again few in number), and the basidiocarp with its component parts. The oospore, the ascus, the teliospore, and the basidium are not fructifications, but in each case merely a component part of a perfect fructification.

If the reasoning presented above is accepted, it becomes necessary to conserve *Cystopus* de Bary vs. *Cystopus* Blume (4). It is so proposed. Concerning *Milesina* Magnus vs. *Milesia* F. B. White no conservation is necessary since *Milesina* conforms with the Rules, while *Milesia* as published was a *Nomen provisiorum.*

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CONCERNING THE CONSERVATION OF THE NAME RHODOPHYLLUS

Rogers (Farlowia **3**: 474–5) is not in favor of conserving this name against *Acurtis, Entoloma,* etc., on the grounds that it is superfluous, and he doubts that conserving it would stabilize nomenclature or avoid disadvantageous changes. He rejects the name *Acurtis* as being based on an "imperfect state." The genus *Acurtis* was based on the carpophoroid stage of *Rhodophyllus abortivus.* Rogers apparently assumes that the "perfect state" in Basidiomycetes is the basidium. I have a letter from Singer dated Jan. 31, 1940, which he states that "Acurtis *does* form basidiospores in some individuals." This evidence, is sufficient to vitiate Rogers' reason for rejecting *Acurtis.*

However, I am not willing to accept the thesis that the basidium itself is the perfect state in the gill fungi. At present the rules read "The perfect state is that which ends . . . in the basidium in the Basidiomycetes. . . ." This clearly applies to the *fruiting stage* in the gill fungi. As far as this group of fungi is concerned nothing could be clearer, and any proposed changes in article 57 which might confuse the situation in this group should not be accepted by the coming Congress. Let us consider what a failure to conserve *Rhodophyllus* will mean:

1. For those who believe, as I do, in the current broad concept of *Rhodophyllus,* all pink-spored agarics with angular spores will have to be transferred to *Acurtis.* This will mean several hundred new combinations.
2. Those who are willing to disregard the name *Acurtis,* but who still use the broad concept of the genus will be forced to

use *Entoloma* and if so, only about two hundred new transfers will be necessary.

3. Those who wish to maintain most of the Friesian groups intact as genera, but who believe that species with smooth longitudinally striated spores (*Clitopilus*) are generically distinct from the angular spored species with decurrent gills (*Acurtis*), will have to use *Acurtis* if they follow the rules and thus introduce *another* generic name, and one which has never been used, in the gill fungi. To me this is clearly a disadvantageous change.

It seems to me that all of Rogers' reasons for voting against the conservation of the name *Rhodophyllus* have been effectively answered. Further discussion would merely belabor the question. In summary, however, it might not be out of order to mention the advantages to be gained by conserving the name.

1. Those who use the broad concept of the genus have an admirably descriptive name which is now in use by the majority of specialists in the gill fungi.
2. Most of the transfers have already been made so definite stability of names will be attained.
3. It will not be necessary to introduce a previously unused generic name for any of the fungi involved.

It is admittedly most unfortunate that Quélet did not use one of his previously established names when he set up his concept of *Rhodophyllus*. But since the name has gained wide acceptance, in this case it is best to keep it. In the question of *Drosophila* vs. *Psathyrella* the opposite is true since both are in use by specialists, and the proponents of *Drosophila* cannot present a case for its conservation which is parallel to that of *Rhodophyllus*.—ALEXANDER H. SMITH.

CONCERNING THE AUTHORSHIP OF MARSSONIA

The first fungus collection referred to the genus *Marssonia* appeared in Rabenhorst's Fungi europaei exsiccati, no. 1857

(1874) as *Colerua potentillae* (Wallr.) Fr. with the collection note "leg. Fischer (sub nom. '*Marssonia potentillae* Fisch')." The botanist Fischer's given name has never been revealed anywhere in the extensive literature concerning *Marssonia.* Although Magnus (Hedwigia **45**: 88–91. 1906.) later substituted the name *Marssonina* for Fischer's *Marssonia,* the identity of Fischer should be of historical interest. After a series of articles by Arthur (Phytopath. **23**: 559–561; 841. 1933.) and Spalding (Phytopath. **23**: 203–204. 1933.) concerning the authorship of the rust species *Cronartium ribicola,* H. Sydow (Ann. Myc. **32**: 115–117. 1934.) established the author to be Johann Carl (Karl) Fischer, a botanist of Stralsund, Pomerania. The specimens of both *C. ribicola* and *M. potentillae* were collected and named by Fischer at Stralsund and distributed in Rabenhorst's Fungi europaei exsiccati as nos. 1595 and 1857, respectively. In addition, Magnus (1906) depicted the author of *Marssonia* as "der Stralsunder Lehrer Fischer." These and related facts leave no doubt that the author of *Marssonia* was Johann Carl Fischer.—PAUL L. LENTZ.

NOTICE

Dr. Bruno Schussnig, formerly of the University of Vienna, is engaged in preparing manuscript of the second volume of his "Vergleichende Morphologie der Niederen Pflanzen." If you can spare reprints of your mycological papers, please send these to him at his present address: Innsbruck-Igls, Austria.

NEIL EVERETT STEVENS, 1887–1949.

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NEIL EVERETT STEVENS, 1887–1949

C. L. SHEAR

Neil Everett Stevens was born April 6, 1887, in Portland, Me., and died in Urbana, Ill., on June 26, 1949. His ancestors were of English extraction. His father used to refer to the family as "Maineacs." His first ten years were spent at Portland where he attended the public schools. Unfortunately I have no record of this period, which must have been of great importance in determining his character and intellectual development. His early life being confined to the city, he missed the intimate contact with nature and agricultural operations without which the investigator of practical agricultural and horticultural problems is somewhat handicapped.

In 1897 the family moved to Auburn, Maine, where Neil graduated from high school in 1904. His education was continued at Bates College, where he graduated with the degree of A.B. in 1908. He then pursued graduate studies at Yale, receiving the Ph.D. degree in 1911. That he must have been above the average in his class is indicated by his election to Phi Beta Kappa and Sigma Xi. He took his collegiate honors lightly. He had no P.B.K. key until recently, when his family made him a present of one.

In 1914 he married Maude Bradford, also from Maine, who was his faithful helpmeet and companion to the end. There are three children, twin sons, Russell Bradford (Ph.D. Wisconsin), associate professor of botany, University of Tennessee; Carl Mantle

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2nd (Ph.D. Illinois), associate professor of chemistry in the State College of Washington, and Mary Christine, the wife of Lt. James McCray, U. S. Army.

He was a member of the following organizations: American Phytopathological Society, vice-president 1933, president 1934; Botanical Society of Washington, secretary 1928, president 1931; American Association for the Advancement of Science Council 1933; Mycological Society of America Council 1932; University Club, Urbana; Botanical Society of America, president 1948.

Neil, as his friends and coworkers familiarly addressed him, was my closest friend and associate in pathological and mycological investigation in the Bureau of Plant Industry for 24 years (1912–36). After so long and intimate an association one could hardly be expected to be satisfied with a mere formal record of the principal events of his life and work. Hence I hope to be pardoned if I indulge in some reminiscences of a more personal nature. Cut down so suddenly while very active and productive, his death came as a great shock to all who knew him. While attending the meeting of the Botanical Society of America at Chicago in 1948 as retiring president, he was stricken with heart disease but after some time in the hospital he was able again to undertake some of his University work. He retained much of his usual zest and cheerfulness. A quotation from his last letter to me in March 1949 is characteristic. He said: "Your letter of Feb. 3 was what I needed. I had been thinking for the last three weeks! I bet C.L.S. will want me to go up to Katahdin with him this summer and I don't feel like it. Now, however, that you don't feel like it either, that makes it all right. About 6 A.M., January 3, I did think the old gentleman with the scythe had come for me. However, I decided that I had already had twice as much fun in this life as any one was entitled to, which took some of the sting out. However, he decided otherwise and I have spent a month in bed. Honestly one of the happiest months you can imagine."

This indicates his general attitude toward life.

"After reading and discussing the various attempts of scientists and philosophers to solve some of the eternal mysteries of man and the universe, we came to the conclusion that the problem is beyond the capacity and comprehension of the human mind in its present stage of development and that we must be content to contribute a few additions to our present fund of human knowledge and make such practical applications as are possible for the improvement of our physical and social condition, with faith in the belief that the practice of the golden rule will finally bring peace and happiness to the human race."

In one of his latest articles (Amer. Scientist **37**: 119. 1949) he explains his attitude toward scientific investigation and his method of approach, closing with approval the words of R. L. Stevenson: "To travel hopefully is a better thing than to arrive, and the true success is to labor."

The breadth and diversity of Neil's intellectual interests are well illustrated in a series of papers collected and published by Dr. Verdoorn in Chronica Botanica 1947 under the title, *Factors in Botanical Publication and Other Essays,* and in the rather complete bibliography appended.

As to religion, he was a member of the Methodist Church. His practical application of religion in daily life was humanitarian rather than creedal. His liberalism in theological matters is well expressed in an article on Dean Inge's views on science and religion (Science **63**: 281. 1926) in which he quotes with evident approval the following statement of the Dean: "I believe that in Science has come the chief revelation of the will and purposes of God that has been made to our generation."

He was very fond of outdoor life and exercise and as a member of the Appalachian Trail Club spent many of his holidays in breaking trails.

Though much interested in mycology and its history, his principal work was in plant pathology and especially cranberry diseases and related problems. His numerous publications in this field were of much scientific value and of great practical application. This work was greatly appreciated by cranberry growers everywhere. A very appreciative sketch of Dr. Stevens was published in "Cranberries" for December 1941 by the editor C. J. Hall.

He followed Dr. C. E. Bessey's advice to his botanical students: "Keep your mind in meristem condition." He was alert and ready to absorb new knowledge and grow. He always approached a problem with an open mind and after reviewing all that had been published on the subject began observations and experiments to test previous conclusions and theories and to evolve new ones. He was no blind worshiper of authority. His studies in the history of science had shown how frequently the supposedly well established theories and conclusions of one generation may be replaced by the next and that absolute final conclusions can never be reached by mortal man; but that the joy of seeking and the pleasure of discovering something apparently new or useful was his greatest reward.

His first contribution to botanical literature was *Discoid Gemmae in the Leafy Hepatics of New England*, published in 1910 while he was doing graduate work under Dr. Evans of Yale. His next was in 1911 on *Dioecism in the Trailing Arbutus*. His Doctor's thesis was *Observations on Heterostylous Plants* published in 1912.

Under the stimulating influence of Dr. Wieland he became much interested in paleobotany, which resulted in a paper on *A Palm from the Upper Cretaceous of New Jersey*. His only mycological and pathological work at Yale was a course in forest pathology under Dr. Graves and his first paper in this field was on *Wood Rots of the Hardy Catalpa*. His broad training in different branches of botany and laboratory technique provided an excellent foundation for later specialization in mycology and pathology and a continued interest in all phases of plant science, both pure and applied.

Soon after graduation at Yale he received an appointment in the Botanical Department of Kansas State College where he taught one year. This experience broadened his views of the country and its agricultural problems. During this period he took a civil service examination for a position in Forest Pathology and to his surprise received an appointment in the Bureau of Plant Industry and was assigned to work on the chestnut blight project. It was here we first met and began working together in an attempt to discover the origin and relationship of the causal organism, *Endothia parasitica*. There was a difference of opinion among the investigators as to whether it was a variety or mutation of one of our native species or a foreign species recently introduced. Our work together on this project as well as all the later work on which we published joint papers was so intimate that the contributions of each cannot be satisfactorily segregated. In general he made most of the cultures and cross inoculation experiments but also considered critically the taxonomic questions relating to the generic and specific concepts involved. We agreed that for practical taxonomic purposes species should be based on morphological, life cycle and cultural differences, which were proved by the comparison of an abundance of specimens from different localities.

It was soon demonstrated by means of specimens obtained from China by Mr. Frank Meyer, the agricultural explorer, and by those from Japan that the blight fungus was common on oriental chestnuts, but not a serious parasite, and that the fungus in this country was undoubtedly brought here on nursery stock. Here it found our native chestnut an easy victim and proceeded to exterminate it throughout the country. The result of this work was a monograph in 1917 of the species of *Endothia* thus far known.

In 1915 he was transferred to the office of fruit disease investigations and began work on strawberry diseases on which he published several papers, especially on the fruit rots.

Two years later he began his studies on cranberry diseases which were carried on in cooperation with the writer and other members of the office. This work he continued with slight interruptions for the remainder of his life, though restricted during his teaching career to his summer vacations. In carrying on the work on cranberries we visited all the cranberry growing districts in the United States and collected fungus and diseased material throughout the natural range of the two species, *Vaccinium macrocarpum* and *V. oxycoccus,* and at the same time all the fungi found on Ericaceous hosts, to determine their distribution and relation to those found on the cranberry. This work led us from the swamps of the Carolina coast to the summits of Mt Washington and Katahdin, through the Adirondacks, Michigan and the Pacific coast of Washington and Oregon. At the same time all the available types and authentic specimens of the fungi reported on *Vaccinium* and closely related genera were studied. This led to the herbaria of Peck, Ellis, Schweinitz, Curtis, Ravenel, Peters, and others. It also naturally led to an interest in the authors themselves and their methods of work, as such knowledge is essential in order to evaluate their results and publications. The search for biographical information led to visits to the homes and collecting grounds of the mycologists mentioned and to the accumulation of much information and many specimens. This work resulted in the publication of several biographical sketches (**12–16, 29, 32**) and a deep and permanent interest in the history of mycology in general and especially in America. A large collection of notes and letters have been accumulated from which we had hoped to prepare a history of mycology in America. In one of his last letters in reply to one in which I had urged him to retire and take up the history we had so long planned, he replied "We may get to that history of mycology yet."

In 1924 he began studies of *Botryosphaeria, Physalospora* and their pycnidial forms, *Dothiorella, Sphaeropsis,* and *Diplodia,* with a study of the currant blight and its cause *Botryosphaeria ribis chromogena.* The work on this fungus led to a thorough study of other species of *Botryosphaeria* and the closely related genus *Physalospora* and their life cycles and host relations. This involved cross inoculations and the examination of type material of the numerous species of *Sphaeropsis* and *Diplodia* which have been described. These species have been based largely on the host plant upon which they happened to be found. It was found that *Botrysphaeria* and *Physalospora* differ chiefly in their pycnidial stages, the former having *Dothiorella* and the latter *Sphaeropsis* or *Diplodia.* The results of this work were published in a series of papers (**19–27, 30, 34, 35, 38–40**). The winter of 1927–28 was spent in the Hawaiian Islands collecting fungi and studying fruit diseases. We made extensive collections on Oahu, Maui, Hawaii and Kauai and brought back more than a thousand specimens, mostly Pyrenomycetes. Only a small part of these have yet been studied although a joint paper (**27**) on the species of *Botryosphaeria* and *Physalospora* found was published.

In 1930 he went to England as a delegate to the International Botanical Congress at Cambridge, and studied strawberry and other fruit diseases and fungi in England, Scotland and Wales.

Some of the results of this work were published in a paper (**35**) on two Physalosporas found in England, thus clearing up the confusion in the life cycles of *Sphaeropsis malorum* Peck and of *S. malorum* Berk.

From 1930–33 he was in charge of the Plant Disease Survey in which he did much excellent work in improving methods of estimating crop losses from diseases and forecasting disease occurrence.

During 1934–35 he worked in the office of Cereal Crops and Diseases studying bacterial wilt of sweet corn. From 1931–36 he also acted as adjunct professor of botany at George Washington University. In 1935 he was a delegate to the International Botanical Congress in Holland. In 1936 he was offered the position of professor of botany at the University of Illinois. He considered and discussed for some time the question whether he could contribute more to the advancement of science and humanity in the Department or in the University. He finally decided in favor of the University, as it would not only give an opportunity to continue some of his research but also to put into practice some of his ideas for the improvement of college teaching. In spite of his rather youthful appearance and lack of the dignity supposed to characterize the conventional professor he was very popular and his informal manner and enthusiasm stimulated his students.

He was at work at the time of his death with his son Russell on a book entitled *Plant Pathology in Agriculture* which will be completed and published later.

In the premature passing of Dr. Stevens the family has lost a devoted husband and father; his students an inspiring teacher; his friends and acquaintances an enjoyable companion and science and education an able and productive contributor to their advancement.

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THE NEED FOR THE PROBABLE ERROR CONCEPT IN MYCOLOGY *

NEIL E. STEVENS

Two major attempts to recognize the human element in science are well established. It is here proposed that, at least in mycology, the two be combined. First in point of time came the practice of adding to each specific binomial the name of its author. This was a frank, perhaps partly unconscious, recognition of the fact that no two authors would necessarily agree in delimiting a species and thus the binomial could be made meaningful only if its author was always named.

Stripped of the mathematical symbols which render it to some sacred and to others diabolical the probable error concept has the same human, almost humanitarian, basis. This is emphasized by the fact that Bessel, an early great student of probable error, preferred to call it "the personal equation" (1). Here too is a frank recognition of the fact that it is impossible to attain absolute accuracy. The probable error is also an attempt to indicate how reliable the measurements were, that is, how much we may depend on them. Like most statistical analyses it is another attempt to indicate the results which represent the true value which would be obtained if it were possible to make observations on all the individuals or cases. How badly just these aids—concepts—are needed in mycology is readily appreciated by those who have worked with long known, much named species.

The suggestion here made is merely that following the name of a new species there be added—perhaps by the editor of the journal publishing the description—a figure indicating the probability of its being indeed a good species. The exact method to be used

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* This note was received for publication a short time before Dr. Stevens' death. The editor does not approve of being placed in the position of having to "rate" the new species proposed by other mycologists, but does recognize merit in the concept as applied to taxonomy. A. H. Smith.

can well be left to the mycological section of the next International Botanical Congress. Certain considerations are, however, obviously important. For example, the number of specimens examined in preparing the description, *i.e.*, their relation to the number probably existing in the world. Other considerations would be the known difficulty of the fungus group and for the older workers the previous record of the author. A graduate student studying his first fungus and deciding that it must be a new species probably should receive a charitably high rating; one, for example, that would indicate there was one chance in a hundred of his having a genuinely new species.

Taxonomy being as Hall and Clements (2) some years ago pointed out, ". . . the only field of science in which the blunders and banalities of the indifferent and incompetent are respected and perpetuated," much interest would be aroused over the "error indices" of various early workers. To Farlow, for example, who spent a lifetime of application and described some half dozen species, one could almost automatically assign an index indicating very low error.

To Berkeley who in a life devoted to the duties of an English parish, the education of thirteen children of his own, plus the conduct of a boarding school of thirty to thirty-five pupils and to mycology, and who described over five thousand new species the rating would of necessity be somewhat different.

LITERATURE CITED

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CHEMICAL AGENTS FOR THE CONTROL OF MOLDS ON MEATS[1]

Frederick T. Wolf and Frederick A. Wolf

INTRODUCTION

The large amounts of spoilage of meat and meat products by molds during World War II made desirable consideration of the various fungi responsible and attempts to devise means of prevention. Species isolated from meats by previous workers (Bidault, **1921**; Brooks and Hansford, **1923**; Wright, **1923**, **1925**; Yesair, **1929**; Haines and Smith, **1933**; Semeniuk and Ball, **1938**; Empey and Scott, **1939**; Jensen, **1942**) include the following: *Alternaria tenuis, Aspergillus chevalieri, A. clavatus, A. niger, A. repens, A. ruber, Botrytis elegans, B. pellicula, B. rosea, Cladosporium herbarum, Dematium pullulans, Hormodendrum cladosporioides, Monilia sitophila, Monascus purpureus, Mucor lusitanicus, M. mucedo, M. racemosus, Oospora lactis, Penicillium anomalum, P. chrysogenum, P. crustaceum, P. expansum, P. melinii, P. notatum, P. puberulum* and various unidentified species of the asymmetrica-velutina group, *Rhizopus nigricans, Sporotrichum carnis, Thamnidium chaetocladioides* and *T. elegans.* It is evident from these lists that those molds usually present in the air and soil are most commonly responsible for meat decay.

It has long been recognized that temperature and humidity are primary factors in relation to the keeping of meats; hence most efforts at meat preservation have involved such purely physical methods as refrigeration and drying. The present study, however, is restricted to a consideration of chemical preservatives, and the inhibition of mold growth through chemical means. Regulations by the Bureau of Animal Industry limit chemical preservatives of meats sold for human consumption in the United States to the following agents: Common salt, sugar, wood smoke, vinegar, spices, saltpeter, $NaNO_3$, $NaNO_2$ and sodium benzoate.

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[1] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number *274* in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

Any study of chemicals for prevention and control of mold on meats should include firstly basic laboratory studies using a representative sampling of pure cultures of fungi to determine concentrations of the various chemical agents which are inhibitory to the various organisms. Such studies should be followed by toxicological and pharmacological tests, to determine whether the chosen chemicals are safe for human consumption. Finally, technological studies, involving means of application of the chosen chemicals, and large scale tests under diverse environmental conditions of the keeping qualities of meats so preserved, must be made. The requirement that a given chemical shall inhibit growth of a number of different fungi and also be safe for human consumption is difficult of fulfillment and is one which has imposed severe limitations upon the types of chemicals that may be employed. Obviously most of the antifungal materials used in plant disease control or in mildew-proofing of textiles must be eliminated from consideration.

MATERIALS AND METHODS

The molds used in these experiments were isolated from meats and meat products in cold storage at Duke University. Seventeen isolates, belonging to eleven species, were studied. These are as follows:

Penicillium crustosum Thom (7)*
Penicillium cyaneum Bainier and Sartory (3)
Penicillium cyclopium Westling (2, 6, 18, 20)
Penicillium olivino-viride Biourge (1, 16, 17)
Penicillium waksmani Zaleski (19)
Aspergillus niger van Tieghem (8)
Aspergillus sydowi Bainier and Sartory (15)
Cephalosporium sp. (9)
Hormodendrum cladosporioides Bonorden (10)
Thamnidium chaetocladioides Link (11)

We are indebted to Dr. K. B. Raper, Northern Regional Research Laboratory, Peoria, Illinois, for identification of the cultures of *Penicillium.* Stock cultures of the test fungi were maintained on slants of Czapek's agar, at room temperature, and transferred at intervals of 10–14 days.

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* The numbers in parentheses were assigned to the isolates employed.

Two different media were employed in the testing of fungistatic agents. One was Czapek's solution, consisting of $NaNO_3$, 3.0 gm.; KH_2PO_4, 1.0 gm.; $MgSO_4$, 0.5 gm.; KCl, 0.5 gm.; $FeCl_3$, trace; and sucrose, 30.0 gm. per liter. The other medium was devised in an attempt to simulate the conditions which obtain in fresh meats, the nitrogen being supplied as glutamic acid and the pH adjusted to 6.0. This medium contained glutamic acid, 2.0 gm.; KH_2PO_4, 0.3 gm.; K_2HPO_4, 2.7 gm.; $MgSO_4$, 0.5 gm.; and dextrose, 50.0 gm. per liter. The two media thus differ in pH, in nitrogen source, in carbon source, and in buffer capacity.

Since the primary consideration in meat preservation is the prevention of spoilage consequent upon growth of fungi, fungistatic rather than fungicidal techniques were chosen.

In testing, the fungi were grown in 125 ml. Erlenmeyer flasks containing 50 ml. of medium, supplemented with the various antifungal materials. Controls provided in each instance consisted of the basal media without added inhibitory chemicals. Except as otherwise indicated, the chemicals were added to the basal media and sterilized by autoclaving 15 minutes at 15 lb. pressure. The pH of the media (4.5 for Czapek's solution and 6.0 for the glutamic acid basal medium) was not adjusted after addition of the inhibitory materials.

Growth of the molds was recorded after one and two weeks' incubation at room temperature, using an arbitrary scale of 0, +, ++, +++, and ++++ in comparison with appropriate controls. For conciseness, only data from observations after two weeks are included herein. Concentrations of fungistatic agents are expressed in terms of percentage on a weight basis, in the case of solids, and on a volume basis for liquid materials.

The findings in the present study are based upon a total of over 9000 cultures.

LITERATURE SURVEY OF CHEMICAL AGENTS

The present experiments include tests of 31 different chemicals. No claims are made that all are safe for human consumption, but, insofar as information is available, this consideration governed the choice of materials to be tested.

It was shown by Kiesel (**1913**), using *Aspergillus niger,* that acetic acid is quite toxic to fungi. Schönberg (**1943**) states that the Germans extensively employed acetic acid in meat preservation during World War II, and his experiments indicate that exposure for 10 minutes to 1.5–2.0% acetic acid will kill spores of *Penicillium glaucum, Aspergillus niger, Cladosporium herbarum* and *Mucor racemosus.* Levine and Fellers (**1940**) studied the influence of pH upon toxicity of acetic acid to *Aspergillus niger.* Sodium acetate was used for purposes of comparison. Sodium diacetate, $(CH_3COONa)_x \cdot (H_2O)_z$, a double salt of sodium acetate and acetic acid, was tested herein because of previous work of Glabe (**1942**), who employed it for prevention of mold and rope-formation in bakery products.

Considerable attention has been devoted to propionic acid and its salts upon growth of fungi. Kiesel (**1913**) reported that 0.0525–0.0586% propionic acid is toxic to *Aspergillus niger.* According to Desgrez and Saggio (**1907**), this acid is relatively non-toxic to animals, the lethal dose for rabbits by the intravenous route being 450 mg. per kilo. Peck and Rosenfeld (**1938**) report that growth of *Trichophyton gypseum, Epidermophyton inguinale,* and *Candida albicans* in Sabouraud's broth is inhibited by propionic acid in concentrations of 0.03%, 0.1%, and 1.0% respectively. Hoffman, Schweitzer and Dalby (**1939**) studied the influence of pH on the toxicity of propionic acid to fungi. Olson and Macy (**1940, 1946**) found that propionic acid is far more fungistatic than the sodium or calcium salts and ascribed fungistasis to the undissociated propionic acid molecule.

Calcium propionate is commonly incorporated with bread dough to inhibit growth of molds. In 1942, more than 1600 bakeries were employing propionate, and the number of loaves so treated was expected to exceed two billion (Miller, **1942**). Propionates are also employed to retard molding of cheese (Miller, **1940**), and, by treatment of wrapping papers, to prevent moldiness of butter (Macy and Olson, **1939**).

It was shown by Keeney (**1943**) and Keeney, Ajello, and Lankford (**1944**) that sodium propionate inhibits growth of a large number of human pathogens, the amounts required varying from 0.0125 to 1.25% for the different ones. Satisfactory therapeutic results with 10% sodium propionate in the treatment of tinea pedis, tinea cruris, tinea capitis, thrush, and otomycosis have been reported by Keeney and Broyles (**1943**). Alter, Jones and Carter (**1947**) successfully employed propionates in the treatment of mycotic vulvovaginitis.

Harshbarger (**1942**) fed sodium and calcium propionate to rats at levels corresponding to 1% and 3% of the total food intake, without ill effects. Rowntree (Keeney, **1943**) injected 20 mg. of sodium propionate into rats daily for nearly a year without untoward results. Keeney, Ajello and Lankford (**1944**) found that the highest non-toxic daily dose of sodium propionate to mice by the intraperitoneal route is 5 mg. Walker and Coppock (**1928**) were able to cultivate *Aspergillus niger* on a medium containing calcium propionate as the sole source of carbon, a finding which is of considerable interest in connection with the use of propionates to check mold growth.

Our tests involved, among the higher fatty acids, caprylic acid, undecylenic acid, and their sodium salts. Peck and Rosenfeld (**1938**) reported that 0.03% caprylic acid is inhibitory to *Trichophyton gypseum.* Wyss, Ludwig and Joiner (**1945**) determined inhibitory concentrations of caprylic acid for *Aspergillus niger, Trichophyton interdigitale* and *T. rubrum.* Foley, Herrmann, and Lee (**1947**) studied the influence of pH upon the inhibition by caprylic acid of *T. gypseum.* Sodium caprylate has been successfully used in the treatment of dermatophytosis of the feet (Keeney, Ajello, Lankford, and Mary, **1945**).

The antifungal activity of undecylenic acid has been much studied in recent years, notably by Peck and Rosenfeld (**1938**), using *Trichophyton gypseum,* Rogler and Greathouse (**1940**), using *Phymatotrichum omnivorum,* Wyss, Ludwig and Joiner (**1945**), using a number of saprophytic molds and human pathogens, Foley, Herrmann and Lee (**1947**), using *T. gypseum* and Rothman *et al.* (**1947**), using *Microsporum audouini.* Extensive use has been made of powders and ointments containing undecylenic acid in the treatment of dermatophytosis. Satisfactory therapeutic results have been obtained by several investigators, including Keeney, Ajello, Broyles and Lankford (**1944**), Shapiro and Rothman (**1945**), Hopkins *et al.* (**1946**), and Sulzberger and Kanof (**1947**).

Some of the derivatives of benzoic acid have been studied also, since sodium benzoate was used in meat preservation by our armed forces during World War II (Jensen, **1942**). Perry and Beal (**1920**) reported that *Penicillium glaucum* failed to grow in the presence of 0.25% sodium benzoate. The toxicity of sodium benzoate to rats was tested by Harshbarger (**1942**) who found it toxic at the 3% level but not toxic when fed at the rate of 1% of the total diet.

p-Amino benzoic acid was shown to be fungistatic by Hoffman, Schweitzer and Dalby (**1942**). Its effects upon the growth of *Aspergillus niger, Penicillium roqueforti* and *Byssochlamys fulva* under varying conditions of pH and nitrogen supply have recently been investigated by Cavill and Vincent (**1945**, **1948**). p-Hydroxy benzoic acid was noted by Uppal (**1926**) to be toxic to *Phytophthora colocasiae*, and it was studied in comparison with the methyl and butyl esters of this acid. The esters of p-hydroxy benzoic acid, according to Neidig and Burrell (**1944**) and Jacobs (**1944**), were used as preservatives of meat products and other foodstuffs in Germany, and are employed by geneticists in Drosophila cultures to suppress mold growth. The methyl and ethyl esters have been used for treatment of dermatophytosis by Bang (**1937**) and Holm (**1944**). The toxicity of these esters to animals is extremely low, the lethal dose being of the order of 3–6 gm. per kilo (Neidig and Burrell, **1944**).

Myers (**1927**) found that thymol, carvacrol, and a number of essential oils, including oil of cloves, are fungicidal. Kingery and Adkisson (**1928**) reported thymol 1:7500 to be fungistatic to a number of dermatophytes, to *Sporotrichum schenckii* and to *Coccidioides immitis*. Our experiments therefore included thymol, carvacrol, oil of cloves, and "Stabilizer No. 1," the latter a mixture of mono isopropyl meta and para cresols, intended for use as a mold inhibitor for starches used in the sizing of paper. A study of oral toxicity of "Stabilizer No. 1" with experimental animals indicated an LD_{50} of 1.2 gm. per kilo (Horsey, **1948**).

Three quinone derivatives were tested, among them, 2, 3, 5, 6 tetrachloro 1, 4 benzoquinone, or "Spergon," an agricultural fungicide used to protect germinating seeds of various crop plants from fungus attack. The LD_{50} of this compound for rats by the oral route was found to be 4 gm. per kilo (McGavack, Boyd, Terranova, and Lehr, **1943**). McNew (**1948**), studying the toxicity to swine of corn treated with tetrabenzoquinone, found that treated seed did not adversely affect animals even when they were maintained on it as long as 117 days. The antifungal activity of 2, 3 dichloro 1, 4 naphthoquinone against 22 species of fungi was investigated by Ter Horst and Felix (**1943**). Woolley (**1945**) tested the effect of this compound upon the growth of bacteria, *Saccharomyces cerevisiae* and *Endomyces vernalis*. Tests made by workers at the New York Medical College in 1943 disclose an LD_{50} to albino rats by the oral route of 3.5 gm. per kilo. Colwell and McCall (**1946**) noted that 2 methyl 1, 4 naphthoquinone is toxic to *Aspergillus niger*. Studies of its toxicity to experimental animals were made by Molitor and Robinson (**1940**).

Various acids, including boric, malonic, crotonic, sorbic and salicylic acids, have been examined in the present study. Boric acid is among the preservatives used in the control of molds of meats by our armed forces during World War II, according to Jensen (**1943**). Malonic acid, which suppresses respiration through its action upon the enzyme succinic dehydrogenase, was included because of studies on *Neurospora* by Ryan, Tatum and Giese (**1944**). Gooding (**1945**) suggested the use of crotonic acid or sorbic acid as a mold inhibitor in foods. Salicylic acid is well known as an antifungal material, and is used in treatment of superficial fungus infections.

Sodium nitrite, an ingredient used in solutions for curing meats, is the only component of such solutions with appreciable antifungal activity.

The compound 2, 4, 5 trichlorophenyl acetate is an agricultural fungicide for use to control cotton anthracnose fungus, *Glomerella gossypii*, on cotton seed. Its LD_{50} for mice is approximately 1.3

gm. per kilo, and this material did not interfere with growth of mice when added to the diet at the rate of 2% (Horsey, **1948**).

"G-4" is 2, 2'dihydroxy 5, 5'dichloro diphenyl methane, and is used in the mildewproofing of canvas, tenting, and other fabrics. Hexamethylene tetramine, according to Jacobs (**1944**), has been employed medically in the treatment of urinary tract infections, and in Germany is a permissible food preservative.

RESULTS

The results, as presented in tables 1–VIII, give the lowest concentration of the various chemical agents which inhibit growth of the test fungi. When one or more organisms were suppressed by the lowest of a series of concentrations tested, the result is reported as less than the concentration stated. Similarly, when one or more molds grew in the highest concentration tested, the result is expressed as more than the stated concentration. Limits were imposed in some cases by the solubility of the chemical agents. From

TABLE I

PERCENTAGE CONCENTRATIONS OF ACETIC ACID AND CERTAIN SALTS REQUIRED FOR FUNGISTASIS

Molds	Concentration				
	Acetic acid		Sodium acetate	Sodium diacetate	
	Czapek	Glutamic	Czapek	Czapek	Glutamic
P. crustosum (7)	0.2	0.25	18	<0.5	0.75
P. cyaneum (3)	0.15	<0.15	15	<0.5	<0.5
P. cyclopium (2)	0.2	0.25	18	<0.5	0.75
P. cyclopium (6)	0.2	0.25	18	<0.5	0.75
P. cyclopium (18)	0.2	0.25	>18	0.75	0.75
P. cyclopium (20)	0.2	0.25	18	0.75	0.75
P. martensii (4)	0.15	<0.15	18	<0.5	<0.5
P. martensii (12)	0.15	0.25	18	<0.5	<0.5
P. olivino-viride (1)	0.15	0.25	18	0.75	0.75
P. olivino-viride (16)	0.15	0.25	>18	0.75	0.75
P. olivino-viride (17)	0.15	0.2	15	<0.5	<0.5
P. waksmani (19)	<0.1	0.25	18	<0.5	<0.5
A. niger (8)	0.2	0.25	18	<0.5	0.75
A. sydowi (15)	<0.1	0.25	>18	0.75	0.75
Cephalosporium sp. (9)	<0.1	<0.15	15	<0.5	<0.5
H. cladosporioides (10)	<0.1	<0.15	9	<0.5	<0.5
T. chaetocladioides (11)	0.2	0.25	9	0.75	0.75

a practical standpoint, special significance is attached to the lowest concentration inhibiting growth of all the test organisms.

Acetic acid (TABLE I) inhibits growth of all the fungi tested in a concentration of 0.2% on Czapek's medium, and 0.25% on the glutamic acid medium. For comparative purposes, tests were made with sodium acetate. None of the molds was inhibited by concentrations of less than 9% sodium acetate, and three were not inhibited by 18%. These findings indicate that the acetate ion is virtually non-toxic to these fungi, and that a large part of the toxicity of acetic acid may be ascribed to the non-ionized acetic acid molecule.

TABLE II

INHIBITION OF MEAT MOLDS BY PROPIONIC ACID AND ITS SALTS

Molds	Concentration					
	Propionic acid		Sodium propionate		Sodium propionate	Calcium propionate
	Czapek	Glutamic	Czapek	Glutamic	Czapek sugar	Czapek
P. crustosum (7)	0.15	0.2	12	7	8	5
P. cyaneum (3)	<0.1	0.15	4	3	6	3
P. cyclopium (2)	0.15	0.2	10	7	12	>6
P. cyclopium (6)	0.15	0.2	12	7	12	3
P. cyclopium (18)	0.15	0.2	12	5	>12	>6
P. cyclopium (20)	0.15	0.2	12	7	12	>6
P. martensii (4)	<0.1	0.15	10	5	10	3
P. martensii (12)	<0.1	0.15	10	5	8	>6
P. olivino-viride (1)	<0.1	0.15	10	5	8	>6
P. olivino-viride (16)	<0.1	0.15	10	5	>12	>6
P. olivino-viride (17)	0.15	0.15	12	5	10	>6
P. waksmani (19)	<0.1	0.15	10	5	8	3
A. niger (8)	<0.1	0.15	4	5	6	3
A. sydowi (15)	<0.1	0.15	10	5	12	5
Cephalosporium sp. (9)	<0.1	<0.1	4	3	6	<1
H. cladosporioides (10)	<0.1	<0.1	4	3	6	<1
T. chaetocladioides (11)	<0.1	0.15	6	5	>2	<1

The sample of sodium diacetate tested was stated by the manufacturer to have an acetic acid content of 33–35%. This agent was inhibitory to all molds in a concentration of 0.75%. Comparison of this result with those obtained with acetic acid and with sodium acetate indicates that the inhibitory action of sodium diacetate is due to its acetic acid content.

Propionic acid (TABLE II) is slightly more toxic to molds than is acetic acid, concentrations inhibitory to all organisms being 0.15% on Czapek's medium and 0.2% on glutamic acid medium. The molds used in the present work are far more resistant to propionates than are the human pathogens. A concentration of 4% sodium propionate was required to inhibit the more sensitive meat molds on Czapek's medium, while the concentration had to be increased to 12% to inhibit the more resistant organisms. Sodium propionate was found to be more effective on the glutamic acid medium than on Czapek's medium, a concentration of 7% sodium propionate checking all mold growth on the glutamic acid medium. These results, apparently anomalous in relation to the initial pH of the two basal media without added propionate, may be interpreted as due to a shift in pH of the media owing to the addition of an "alkaline" salt, and its effect upon the ionization constant of propionic acid. Calcium propionate proved more toxic to the test fungi than did sodium propionate. The calcium salt is, however, so insoluble that a 6% concentration, approaching saturation, was insufficient to check growth of seven of the test organisms.

In attempting to evaluate the significance of propionates as a carbon source for fungi in relation to fungistasis, Czapek's medium, lacking sugar, was prepared and varying amounts of sodium propionate were added as a sole source of carbon. Resultant growth indicated that all of the molds, with the single exception of *Thamnidium chaetocladioides,* are able to utilize propionate. Sodium propionate is far inferior to sucrose as a carbon source for these fungi, however, since growth of the +++ and ++++ categories was not obtained in any instance upon propionate, regardless of its concentration. The inhibitory concentrations of sodium propionate in Czapek's medium lacking sucrose compare favorably with those obtained in the same medium containing sucrose. The fact that fungi are able to utilize propionate as a carbon source appears to offer at least a partial explanation of the large amounts required to inhibit their growth.

Caprylic acid (TABLE III) is more toxic to meat molds than the fatty acids of lower molecular weight, the concentrations required for suppression of all growth being 0.05% on Czapek's medium

TABLE III

CONCENTRATIONS OF CAPRYLIC ACID AND UNDECYLENIC ACID, AND THEIR SALTS REQUIRED FOR FUNGISTASIS OF MEAT MOLDS

Molds	Concentration							
	Caprylic acid		Sodium caprylate		Undecylenic acid		Sodium undecylenate	
	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	0.025	0.075	0.3	0.2	>0.2	0.05	0.05	0.1
P. cyaneum (3)	<0.01	0.05	<0.05	0.1	0.05	0.025	<0.025	<0.025
P. cyclopium (2)	0.05	0.075	0.3	>0.3	>0.2	0.05	0.1	0.1
P. cyclopium (6)	0.05	0.075	0.3	0.3	>0.2	0.1	0.1	0.1
P. cyclopium (18)	0.025	0.075	0.3	0.3	>0.2	0.05	0.1	0.1
P. cyclopium (20)	0.025	0.075	0.3	0.3	>0.2	0.05	0.1	0.1
P. martensii (4)	0.025	0.05	0.3	0.2	0.2	0.05	0.05	0.05
P. martensii (12)	0.05	0.075	0.1	0.2	>0.2	0.05	0.1	0.1
P. olivino-viride (1)	0.05	0.05	0.3	0.3	>0.2	0.025	0.1	0.05
P. olivino-viride (16)	0.025	0.075	0.1	0.2	>0.2	0.05	0.05	0.05
P. olivino-viride (17)	0.025	0.075	0.3	0.2	>0.2	0.05	0.05	0.1
P. waksmani (19)	0.05	0.075	0.3	0.3	>0.2	0.05	0.05	0.1
A. niger (8)	0.05	0.075	0.3	0.3	>0.2	0.05	0.1	0.05
A. sydowi (15)	0.05	0.075	0.05	0.3	0.05	0.05	0.1	0.1
Cephalosporium sp. (9)	<0.01	<0.025	<0.05	<0.05	0.05	0.025	<0.025	<0.025
H. cladosporioides (10)	<0.01	<0.025	<0.05	<0.05	<0.01	0.025	<0.025	<0.025
T. chaetocladioides (11)	0.025	0.075	0.1	0.1	>0.2	>0.1	0.1	0.1

and 0.075% on the glutamic acid medium. Tests with sodium caprylate gave a value of 0.3% on both media.

Experiments with undecylenic acid in Czapek's medium gave no satisfactory mold control at any concentration tested, a result attributable to the very low solubility of this material in water under conditions of low pH. With this acid in the glutamic acid medium, growth of all except two of the test organisms was checked at a concentration of 0.05%. Sodium undecylenate added in the amount of 0.1% inhibited all fungi on both media.

A concentration of 1.5% sodium benzoate (TABLE IV) was required to suppress growth of all the meat molds, so that this material is a rather poor fungistatic agent. Using p-amino benzoic acid, 0.5% was required on Czapek's medium and 1% on glutamic acid medium. p-Hydroxy benzoic acid was ineffective against certain of the molds at a concentration of 1.5%, the limit of its solubility. The esters of p-hydroxy benzoic acid, however, are efficient fungistatic agents, the methyl ester being inhibitory to all the molds tested on both media at a concentration of 0.1%. The butyl ester is the more toxic, but is sufficiently insoluble so that a few of the molds can grow in the presence of 0.02% p-hydroxy n-butyl benzoate, a concentration which represents the limit of solubility of this compound.

There is little difference in the effectiveness of thymol, carvacrol, "Stabilizer No. 1" and oil of cloves (TABLE V). All are effective in the range of concentrations of 0.025–0.05%.

The three quinone derivatives, tetrachlorobenzoquinone, dichloronaphthoquinone, and 2-methyl naphthoquinone, are not heat stable and so were sterilized by Berkefeld filtration. Because these compounds are only sparingly soluble in water, they were dissolved in minimal quantities of 95% alcohol. Tetrachlorobenzoquinone (TABLE VI) was found inhibitory to growth of all except three test organisms at a concentration of 0.005% in Czapek's medium and 0.01% in the glutamic acid medium. Essentially similar findings were obtained with dichloronaphthoquinone. Mold control was obtained with 0.01% 2- methyl naphthoquinone in Czapek's medium, but this concentration, the limit of its solubility, failed to inhibit most of the meat molds on the glutamic acid medium.

TABLE IV

CONCENTRATION OF BENZOATES AND FUNGISTASIS OF MOLDS ON MEATS

Molds	Concentration								
	Sodium benzoate	p-Amino benzoic acid		p-Hydroxy benzoic acid		p-Hydroxy methyl benzoate		p-Hydroxy n-butyl benzoate	
	Czapek	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	1.5	0.5	1.0	1.25	>1.5	0.1	<0.05	>0.02	0.02
P. cyaneum (3)	1.25	0.25	0.75	1.25	>1.5	0.05	<0.05	0.02	<0.01
P. cyclopium (2)	1.5	0.5	1.0	1.25	>1.5	0.05	0.1	0.02	0.02
P. cyclopium (6)	1.5	0.5	1.0	1.25	>1.5	0.05	0.1	>0.02	0.02
P. cyclopium (18)	1.5	0.5	1.0	1.25	>1.5	0.1	0.1	0.02	0.02
P. cyclopium (20)	1.5	0.5	1.0	1.25	>1.5	0.1	0.1	>0.02	0.02
P. martensii (4)	1.0	0.5	1.0	1.25	>1.5	0.1	0.1	0.02	>0.02
P. martensii (12)	1.25	0.5	0.75	1.25	>1.5	0.1	0.05	0.02	>0.02
P. olivino-viride (1)	1.5	0.25	<0.5	1.25	>1.5	0.05	<0.05	<0.01	<0.01
P. olivino-viride (16)	1.5	0.5	<0.5	1.25	1.5	0.05	<0.05	<0.01	0.01
P. olivino-viride (17)	1.5	0.25	1.75	1.25	>1.5	0.05	<0.05	<0.01	<0.01
P. waksmani (19)	1.5	0.25	0.75	>1.5	>1.5	0.1	0.1	>0.02	0.02
A. niger (8)	1.5	0.5	0.75	>1.5	>1.5	0.1	0.1	>0.02	0.02
A. sydowi (15)	1.25	0.25	1.0	>1.5	>1.5	0.05	0.01	<0.01	0.02
Cephalosporium sp. (9)	<0.5	0.25	<0.5	<0.5	<0.5	0.5	0.05	>0.02	0.02
H. cladosporioides (10)	<0.5	<0.1	<0.5	<0.5	<0.5	0.05	0.05	0.02	<0.01
T. chaetocladioides (11)	1.0	0.5	1.0	1.25	>1.5	0.1	0.1	<0.01	<0.01

TABLE V

INHIBITION OF MEAT MOLDS BY THYMOL AND RELATED OILS

Molds	Concentration							
	Thymol		Carvacrol		Stabilizer No. 1		Oil of cloves	
	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	0.02	<0.025	<0.025	0.05	0.02	0.05	0.05	0.05
P. cyaneum (3)	<0.01	0.05	<0.025	<0.025	0.035	<0.035	0.05	0.05
P. cyclopium (2)	0.02	0.05	>0.05	0.05	0.035	0.05	0.05	0.05
P. cyclopium (6)	0.035	0.05	0.05	0.05	0.035	0.05	0.05	0.05
P. cyclopium (18)	0.035	0.05	0.05	0.05	0.035	0.05	0.05	0.05
P. cyclopium (20)	0.035	0.05	0.05	0.05	0.035	0.05	0.05	0.05
P. martensii (4)	0.025	0.05	>0.05	0.05	0.025	0.05	0.05	0.05
P. martensii (12)	0.025	0.05	<0.025	0.05	0.02	0.05	0.05	0.05
P. olivino-viride (1)	0.02	0.05	0.05	0.05	0.035	<0.035	0.05	<0.025
P. olivino-viride (16)	0.02	0.05	<0.025	<0.025	0.02	<0.035	0.05	0.05
P. olivino-viride (17)	0.02	0.05	<0.025	0.05	0.025	0.05	0.05	<0.025
P. waksmani (19)	0.02	0.05	0.05	0.05	0.035	0.05	0.05	0.05
A. niger (8)	0.025	0.05	<0.025	0.05	0.035	0.05	0.05	0.05
A. sydowi (15)	<0.01	0.05	<0.025	<0.025	0.02	<0.035	0.05	0.05
Cephalosporium sp. (9)	0.02	0.05	<0.025	0.05	0.025	<0.035	0.05	0.05
H. cladosporioides (10)	<0.01	<0.025	<0.025	<0.025	0.02	<0.035	<0.025	0.05
T. chaetocladioides (11)	<0.01	<0.025	<0.025	<0.025	<0.01	<0.035	0.05	0.05

Boric acid (TABLE VII) is a weak fungistatic agent, as two of the test strains were not checked by 2% of this material. Malonic acid failed to check growth of many of the fungi when present in a concentration of 0.7%. Crotonic acid proved to be an effective fungistatic material, the concentrations required being 0.1% in Czapek's medium and 0.15% in glutamic acid medium. Its effectiveness is of the same order of magnitude as that of the closely related compound, sorbic acid, which suppressed growth of all molds in both media in a concentration of 0.1%. Salicylic acid has slightly more fungistatic activity. A concentration of 0.05% in Czapek's medium and 0.1% in glutamic acid medium resulted in complete inhibition of growth.

TABLE VI

CONCENTRATION OF CERTAIN QUINONES AND INHIBITION OF MEAT MOLDS

Molds	Concentration					
	Tetrachloro-benzoquinone		Dichloro-naphthoquinone		2 Methyl naphthoquinone	
	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	0.001	0.01	<0.0005	0.005	0.01	>0.01
P. cyaneum (3)	0.001	0.01	0.001	0.01	0.01	>0.01
P. cyclopium (2)	0.005	0.01	<0.0005	0.005	0.005	>0.01
P. cyclopium (6)	0.005	0.01	<0.0005	0.005	0.01	>0.01
P. cyclopium (18)	0.005	0.01	0.005	0.01	0.01	0.01
P. cyclopium (20)	0.005	0.01	<0.0005	0.005	0.01	0.01
P. martensii (4)	0.001	0.01	0.005	0.01	0.01	>0.01
P. martensii (12)	0.005	0.01	0.001	0.005	0.01	>0.01
P. olivino-viride (1)	0.005	0.01	0.001	0.01	0.005	>0.01
P. olivino-viride (16)	0.005	0.01	0.0005	0.005	0.005	>0.01
P. olivino-viride (17)	0.005	0.01	0.001	0.005	0.005	>0.01
P. waksmani (19)	0.005	>0.01	0.001	0.01	0.01	>0.01
A. niger (8)	0.01	>0.01	<0.0005	0.01	0.01	0.005
A. sydowi (15)	0.005	>0.01	0.001	>0.01	0.01	0.005
Cephalosporium sp. (9)	0.005	0.01	<0.0005	0.005	<0.001	>0.01
H. cladosporioides (10)	0.005	0.01	<0.0005	0.005	0.005	0.005
T. chaetocladioides (11)	0.005	0.01	0.01	0.01	0.01	>0.01

Sodium nitrite (TABLE VIII), the only ingredient of solutions used to cure meats that has appreciable antifungal activity, gave good results at the 0.5% level in tests with Czapek's solution, but 2% was required in glutamic acid medium. Trichlorophenyl acetate proved inhibitory in tests on both media at a concentration of 0.005%. "G-4" prevented growth of the various test organisms at

TABLE VII

FUNGISTATIC CONCENTRATION OF CERTAIN ACIDS FOR MEAT MOLDS

Molds	Concentration									
	Boric acid		Malonic acid		Crotonic acid		Sorbic acid		Salicylic acid	
	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	1	<1.5	0.6	>0.7	0.05	0.15	0.1	0.075	0.05	0.1
P. cyaneum (3)	1.5	2	0.6	>0.7	<0.025	0.1	0.025	0.025	0.05	0.1
P. cyclopium (2)	2	2	>0.7	>0.7	0.05	0.1	0.1	0.075	0.05	0.1
P. cyclopium (6)	<1	<1.5	>0.7	>0.7	0.1	0.15	0.1	0.1	0.05	0.1
P. cyclopium (18)	<1	<1.5	0.6	>0.7	0.05	0.1	0.025	0.1	0.05	0.1
P. cyclopium (20)	2	<1.5	0.6	>0.7	0.1	0.15	0.05	0.1	0.05	0.1
P. martensii (4)	>2	2	0.4	>0.7	0.05	0.1	0.05	0.075	0.05	0.1
P. martensii (12)	>2	>2	0.4	>0.7	0.05	0.1	0.05	0.1	0.05	0.1
P. olivino-viride (1)	<1	<1.5	0.6	>0.7	0.05	0.1	0.1	0.075	0.05	0.1
P. olivino-viride (16)	1.5	<1.5	0.6	>0.7	0.05	0.1	0.025	0.075	0.05	0.1
P. olivino-viride (17)	<1	<1.5	0.4	>0.7	0.05	0.1	0.025	0.075	0.05	0.1
P. waksmani (19)	<1	<1.5	0.6	>0.7	0.05	0.1	0.1	0.1	0.05	0.1
A. niger (8)	2	<1.5	>0.7	>0.7	0.05	0.1	0.1	0.1	0.05	0.1
A. sydowi (15)	2	<1.5	>0.7	>0.7	0.1	0.15	0.1	0.1	0.05	0.1
Cephalosporium sp. (9)	<1	<1.5	0.4	0.4	<0.025	0.1	0.025	0.025	0.05	0.1
H. cladosporioides (10)	1.5	<1.5	<0.2	0.4	<0.025	<0.05	0.025	0.025	0.05	0.1
T. chaetocladioides (11)	<1	<1.5	<0.2	0.4	0.05	0.1	0.025	0.025	0.05	0.1

TABLE VIII

CONCENTRATION OF SODIUM NITRITE AND CERTAIN ORGANIC FUNGICIDES REQUIRED TO INHIBIT MEAT MOLDS

Molds	Concentration							
	Sodium nitrite		Trichlorophenyl Acetate		"G-4"		Hexamethylenetetramine	
	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	0.5	2	0.001	0.005	0.01	<0.005	0.1	<0.05
P. cyaneum (3)	0.1	1	0.005	0.005	>0.01	<0.005	<0.05	<0.05
P. cyclopium (2)	0.25	2	0.001	0.005	0.01	<0.005	0.1	<0.05
P. cyclopium (6)	>0.5	2	0.001	0.005	<0.005	<0.005	0.1	0.1
P. cyclopium (18)	0.5	1	0.001	0.005	>0.01	<0.005	0.15	<0.05
P. cyclopium (20)	0.5	1.5	0.001	0.005	0.01	<0.005	<0.05	<0.05
P. martensii (4)	0.1	0.5	0.001	0.005	0.01	<0.005	0.2	0.2
P. martensii (12)	0.1	1	0.001	>0.005	0.01	<0.005	>0.2	0.15
P. olivino-viride (1)	0.5	1	0.005	0.005	0.01	<0.005	<0.05	<0.05
P. olivino-viride (16)	0.5	2	>0.0005	0.005	>0.01	<0.005	0.15	0.1
P. olivino-viride (17)	0.25	0.5	0.001	0.005	0.01	<0.005	0.15	0.15
P. waksmani (19)	0.5	1	0.005	0.005	0.01	<0.005	<0.05	<0.05
A. niger (8)	0.25	1	0.001	>0.005	0.01	<0.005	<0.05	<0.05
A. sydowi (15)	0.5	1.5	0.005	0.005	0.01	<0.005	0.1	<0.05
Cephalosporium sp. (9)	0.5	0.5	0.001	0.005	0.01	<0.005	<0.05	<0.05
H. cladosporioides (10)	0.1	0.5	0.001	0.005	<0.005	<0.005	<0.05	<0.05
T. chaetocladioides (11)	0.5	2	0.005	0.005	0.01	<0.005	0.15	0.1

a concentration of 0.005–0.01%. Hexamethylenetetramine proved to be inhibitory at a level of 0.2%.

DISCUSSION

The problem of chemical control of molds on meats is complex, involving consideration of concentrations required to achieve fungistasis under different environmental conditions, toxicity to man, absence of appreciable odor or taste, methods of application, and cost. The present report constitutes a preliminary approach to the problem, and the data do not justify a complete answer to the question of which chemical agent is ideal for mold control. As a result of this screening procedure, however, certain of the chemicals tested appear to be well-suited, others may or may not be of value, and others may be eliminated from further consideration.

Acetic acid and sodium diacetate appear especially deserving of further consideration, whereas sodium acetate is obviously worthless. Propionic acid appears to be sufficiently effective as a fungistatic agent, but may be found undesirable because of taste or odor. Sodium and calcium propionates have several distinct disadvantages, notably the large amounts required for prevention of mold growth, the utilization of these compounds as sources of carbon for certain fungi, and the relative insolubility of the calcium salt. These materials, however, may merit further study. Likewise the higher fatty acids, caprylic acid and undecylenic acid, are effective fungistatic agents, although undecylenic acid has the disadvantage of low solubility in water, and both of these materials may be found objectionable because of taste or odor. These difficulties, however, are not encountered with sodium caprylate and sodium undecylenate, which should be considered further.

Although some use was made of sodium benzoate under World War II conditions and its fungistatic activity is much greater than that of the propionates, yet it does not appear to have sufficient antifungal activity to be of marked value. The same is true of p-amino benzoic acid. p-Hydroxybenzoic acid is obviously worthless for use against fungi, but its esters appear to have a number of favorable characteristics, and should definitely be considered for commercial use.

Each of the closely related materials, thymol, carvacrol, "Stabilizer No. 1," and oil of cloves, possesses marked fungistatic activity. Carvacrol and "Stabilizer No. 1," however, should probably be eliminated from further consideration because of their pronounced cresol odor. But thymol and oil of cloves should be tested more thoroughly from the standpoint of toxicity to man before attempts are made to employ them in meat preservation.

Tetrachlorobenzoquinone and dichloronaphthoquinone are sufficiently effective against fungi and nontoxic to man to merit further consideration. A serious disadvantage possessed by 2-methyl naphthoquinone is its sensitivity to light.

Boric and malonic acids do not appear to be sufficiently active against molds to be of appreciable value, notwithstanding the limited success obtained with boric acid during World War II. Crotonic and sorbic acids gave excellent results in the present experiments, and should be given further trials. It is felt that salicylic acid, while effective as an antifungal agent, should be further studied from a pharmacological standpoint.

Sodium nitrite does not possess sufficient fungistatic power to make it useful in preventing moldiness. Trichlorophenyl acetate appears to be an excellent inhibitor of fungus growth, while "G-4," like 2-methyl naphthoquinone, is inactivated in the presence of light. Hexamethylene tetramine may be worthy of further trial.

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SUMMARY

These experiments constitute attempts to evaluate 31 chemical agents for the control of mold spoilage of meats. The test fungi include *Penicillium crustosum, P. cyaneum, P. cyclopium, P. martensii, P. olivino-viride, P. waksmani, Aspergillus niger, A. sydowi, Cephalosporium* sp., *Hormodendrum cladosporioides* and *Thamnidium chaetocladioides*. The fungistatic concentration of each chemical agent has been determined for each test organism upon two different media.

It is concluded that acetic acid, sodium diacetate, sodium caprylate, sodium undecylenate, methyl p-hydroxy benzoate, n-butyl p-hydroxy benzoate, thymol, oil of cloves, tetrachlorobenzoquinone, dichloronaphthoquinone, crotonic acid, sorbic acid, and trichlorophenyl acetate possess greatest merit among the antifungal materials tested.

Propionic acid, sodium propionate, calcium propionate, caprylic acid, undecylenic acid, sodium benzoate, p-amino benzoic acid, carvacrol, "Stabilizer No. 1," salicylic acid, "G-4," and hexamethylene tetramine, for one reason or other, are not ideally adapted for control of molds on meats. Sodium acetate, p-hydroxy benzoic acid, 2-methyl naphthoquinone, boric acid, malonic acid, and sodium nitrite, however, appear worthless as fungistatic agents against molds on meats.

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A DACTYLELLA WITH CONIDIA RESEMBLING THOSE OF DACTYLELLA STENOBROCHA IN SIZE AND SHAPE

Charles Drechsler [1]

(with 1 figure)

Several maizemeal-agar plate cultures, which, after being overgrown with mycelium of *Pythium mamillatum* Meurs, had been further planted with small quantities of leaf mold collected in deciduous woods near Newport News, Virginia, on October 15, 1948, became abundantly infested in about ten days with free-living nematodes as well as with different species of *Amoebae* and testaceous rhizopods. In all the cultures the eelworms then began to suffer losses from the capture and destruction of many individuals by the predacious hyphomycete I described earlier (2: 508–513) as *Dactylella gephyropaga*. After this hyphomycete had extended its mycelium throughout the substratum it continued for weeks to destroy eelworms and to produce conidia on its sparsely scattered conidiophores. Two of the cultures on being examined 35 days after the leaf mold had been added showed, besides, in areas near the deposits of forest detritus some development of erect conidiophores that bore solitary conidia manifestly alien not only to *Dactylella gephyropaga* but also to all congeneric species hitherto made known. During the ensuing weeks the new fungus gradually spread over both of the aging cultures, putting forth additional conidial apparatus rather sparingly though in ample quantity for study.

The colorless procumbent mycelial filaments (fig. 1, *A–D: a*) from which the erect conidiophores (fig. 1, *A–D: b*) of the new

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fungus arose were of moderate width for members of the predacious series, and like the hyphae of allied forms were divided at moderate intervals by commonplace cross-walls. The conidiophores similarly offered little distinctiveness. As their height did not usually exceed 400 μ, they appeared in general a little shorter than the intermixed conidiophores of *Dactylella gephyropaga*. From a comparison of measurements they would seem, perhaps, on the whole, somewhat shorter also than the conidiophores of my *Dactylella stenobrocha* (5) and my *Dactylella heterospora* (3), though the dimensional differences concerned here are not impressive for descriptive purposes. While the conidiophores of the new fungus, as a rule, were simple (FIG. 1, *A–C: b*) and regularly produced a single conidium (FIG. 1, *A, c*) at the tip, in occasional instances (FIG. 1, *D, b*) they gave off a lateral branch (FIG. 1, *D, c*) from which a second conidium would be abjointed. When eventually a denuded conidiophore fell onto the substratum it would frequently give rise to a secondary conidiophore from one of its living segments. Through repetition of the same developmental sequence conidiophores of the third and fourth orders were brought into being.

The conidia thus formed aloft sparingly were mostly of an elongated ellipsoidal or strobiliform shape, thereby showing rather marked similarity to the conidia of *Dactylella stenobrocha*, which, indeed, they further resembled closely in their main dimensions. With respect to shape and dimensions they bore notable resemblance also to the larger type of conidia produced by *Dactylella heterospora*, though they lacked the abruptly truncate basal profile so characteristic of the latter. They were decisively distinguished, however, from the conidia of both *D. stenobrocha* and *D. heterospora* by more abundant septation, as they contained from 1 to 9 cross-walls. The specimens showing one septum (FIG. 1, *E, a, b*), two septa (FIG. 1, *F, a–f*), and three septa (FIG. 1, *G, a–h*)—the entire range of septation in *D. stenobrocha*—included only about one-seventh of the conidia produced by the new fungus. Conidia with four (FIG. 1, *H, a–l*), five (FIG. 1, *I, a–j*), six (FIG. 1, *J, a–l*), or seven (FIG. 1, *K, a–p*) cross-walls were present in large numbers, while specimens with eight (FIG. 1, *L, a–b*), or nine

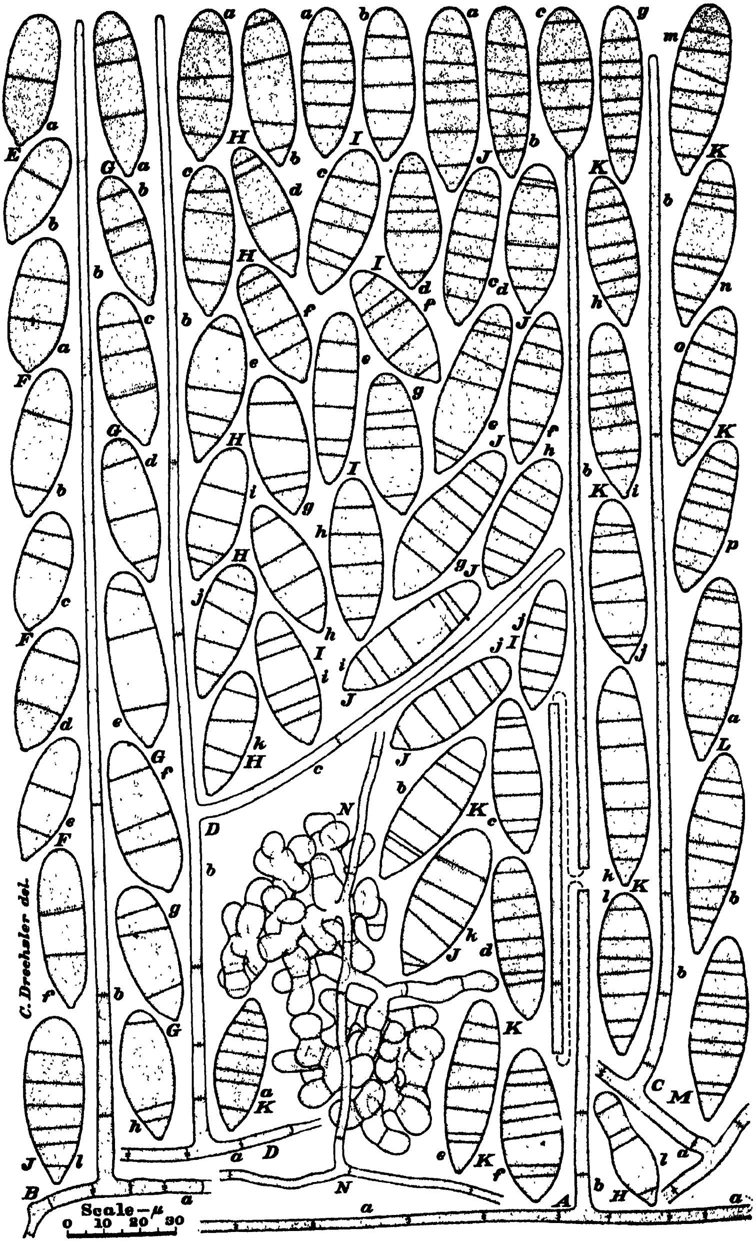

FIG. 1. *Dactylella strobilodes*.

(FIG. 1, *M*) partitions were few. Of 125 conidia taken more or less at random a frequency distribution was noted as follows: 1 septum, 2; 2 septa, 7; 3 septa, 9; 4 septa, 28; 5 septa, 29; 6 septa, 30; 7 septa, 17; 8 septa, 2; 9 septa, 1. The same assortment of conidia gave measurements for length, expressed in the nearest integral number of microns, distributed as follows: 31 μ, 1; 32 μ, 1; 34 μ, 2; 35 μ, 5; 36 μ, 6: 37 μ, 7; 38 μ, 14; 39 μ, 6; 40 μ, 17; 41 μ, 5; 42 μ, 14; 43 μ, 4; 44 μ, 13; 45 μ, 5; 46 μ, 8; 47 μ, 3; 48 μ, 5; 49 μ, 2 ; 50 μ, 4; 52 μ, 1; 55 μ, 1; 60 μ, 1; and measurements for greatest width, expressed in the nearest integral number of microns, with the following distribution: 11 μ, 1; 12 μ, 6; 13 μ, 15; 14 μ, 34; 15 μ, 33; 16 μ, 27; 17 μ, 7; 18 μ, 2.

Several attempts were made to isolate the fungus by removing its conidia from the erect conidiophores to newly poured plates of maizemeal agar—the removal being accomplished, with suitable precautions against contamination, by means of small slabs of sterile agar medium held on a flamed platinum spatula. The conidia in all instances failed to germinate on the sterile agar, though the same materials and procedures have been employed successfully in isolating nearly all of the clampless nematode-capturing hyphomycetes encountered under similar conditions.

As the general habit and morphology of its conidial apparatus left little room for doubt that the fungus was intimately related to the nematode-capturing hyphomycetes, its mycelium both on and under the surface of the agar was examined for some sort of biological relationship. However, I was unable to discover the fungus in any parasitic or predacious relationship either to eelworms or to any other of the several types of minute animals infesting the cultures. Nor could I find it parasitizing *Pythium mamillatum,* or *Dactylella gephyropaga,* or any other fungus that had grown out from the superadded leaf mold into the transparent agar. In a few places submerged filaments were found bearing a somewhat elaborate system of distended, colorless, septate, closely ramified hyphal elements, filled with protoplasm of nearly homogeneous consistency (FIG. 1, *N*). These ramifying systems had much resemblance to the dichotomously branched assimilative apparatus produced by *Dactylella tylopaga* Drechsl. (1) in captured specimens of *Amoeba verrucosa* Ehrenb., but were never found loosely enveloped by any recognizable membrane. Hence there seems little likelihood that the fungus could have subsisted on a pelliculate rhizopod, though the possibility remains that it might have subsisted on a rhizopod devoid of a persistent pellicle. A destructive biological relationship involving a protozoan without any durable membrane—a relationship comparable to that of my *Acaulopage crobylospora* (4) to *Leptomyxa* sp.—might well be difficult of detection.

In respect to its uncertain biological character, the fungus invites comparison with the two allied species I referred earlier to *Dactylella rhombospora* Grove (2: 539–540) and *Dactylaria pulchra* Linder (3: 349–352), as also with the three species I have described under the binomials *Dactylella heptameres* (3: 352–354), *Dactylella rhopalota* (3: 354–357), and *Dactylella atractoides* (3: 357–360). Among the several species that have been described under *Dactylella* in the literature but have so far not developed in my agar cultures, it seems most to resemble *Dactylella minuta* Grove (6). Identity with *D. minuta* seems unlikely, however, as that species has considerably shorter (120 to 150 μ) conidiophores together with generally longer (60 to 70 μ) conidia; these conidia, moreover, to judge from Grove's figures, being of clavate rather than elongated ellipsoidal shape. The fungus from Newport News apparently has no close morphological similarity to any of the forms described in *Monacrosporium*, a genus presumably identical with *Dactylella*. Its description as a new species may advantageously forestall confusion with *Dactylella stenobrocha* and *Dactylella heterospora*. The specific epithet applied to it is intended to be conveniently suggestive in signalizing the resemblance in shape of its conidia to pine cones.

Dactylella strobilodes sp. nov.

Mycelium sparsum; hyphis sterilibus incoloratis, mediocriter septatis, plerumque 2–4.5 μ crassis, ex maxima parte parce ramosis, sed cristas crebre ramosas in ramulis brevibus 4–8.5 μ latis consistentes rarissime ferentibus; hyphis fertilibus incoloratis, erectis, plerumque 3–8 septatis, 250–400 μ altis, basi 4–7.5 μ crassis, sursum leniter attenuatis, apice 2–3 μ crassis, vulgo simplicibus sed quandoque ramo praeditis, in quoque apice conidium gignentibus; conidiis incoloratis, elongato-ellipsoideis vel strobiliformibus, apice late rotun-

datis, basi saepe pediculo obtuso praeditis, 1–9 septatis plerumque 3–7 septatis, 30–60 μ (saepius circa 41.5 μ) longis, 11–18 μ (saepius circa 14.6 μ) crassis.
Habitat in humo silvestri prope Newport News, Virginia.

Mycelium sparse; vegetative hyphae colorless, septate at moderate intervals, mostly 2 to 4.5 μ wide, in general rather sparingly branched but in widely spaced positions bearing closely ramified systems composed of branches often 4 to 8.5 μ in diameter. Conidiophores colorless, erect, often containing 3 to 8 cross-walls, commonly 250 to 400 μ high, 4 to 7.5 μ wide at the base, narrowing gradually upward, mostly 2 to 3 μ wide at the tip, usually simple but occasionally with a lateral branch, at each tip bearing a single conidium. Conidia colorless, elongated ellipsoidal or strobiliform, broadly rounded at the tip, at the base often furnished with a blunt hilar protrusion, divided by 1 to 9 (usually by 3 to 7) cross-walls, measuring mostly 30 to 60 μ (average 41.5 μ) in length and 11 to 18 μ (average 14.6 μ) in width.

Occurring in leaf mold in deciduous woods near Newport News, Virginia.

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EXPLANATION OF FIGURE

FIG. 1. *Dactylella strobilodes*, drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, Prostrate hypha, *a*, from which has been sent up an unbranched erect conidiophore, *b*, that bears terminally a quinqueseptate conidium, *c;* owing to lack of space the conidiophore *b* is shown in three parts whose proper connection is indicated by broken lines. B, C, Portions of prostrate hyphae, a, from each of which

arises an unbranched erect conidiophore, *b*, shown denuded of the single conidium that had been borne on its tip. *D*, Portion of prostrate hypha, *a*, from which has been sent up a conidiophore, *b*, that bears a lateral branch, *c*; the tip of the main hypha, as also that of the branch, is shown denuded of the single conidium that had been borne on it. *E*, Uniseptate conidia, *a* and *b*. *F*, Biseptate conidia, *a–f*. *G*, Triseptate conidia, *a–h*. *H*, Quadriseptate conidia, *a–l*. *I*, Quinqueseptate conidia, *a–j*. *J*, Six-septate conidia, *a–l*. *K*, Seven-septate conidia, *a–p*. *L*, Eight-septate conidia, *a* and *b*. *M*, Nine-septate conidium. *N*, Densely ramifying system of thick lobulate branches found produced on a submerged mycelial hypha.

LEAF BLOTCH OF POPLAR CAUSED BY A NEW SPECIES OF SEPTOTINIA

ALMA M. WATERMAN[1] AND EDITH K. CASH[2]

(WITH 2 FIGURES)

For several years a conspicuous leaf blotch on plantation trees of hybrid poplar clones has been under observation in the eastern United States. The symptoms differ from those resulting from infection by the leaf fungi common on native poplars in that area and the causal organism has only recently been identified as a species of *Septotinia.* This genus was established by Whetzel (**5**) in the subfamily Ciborioideae of the Helotiaceae, based on *S. podophyllina* Whetzel, the only species then known in the genus.

SYMPTOMS

The leaf blotch first appears in early spring as small brown spots on the young developing leaves, frequently at or near the margin, but also at any point on the leaf blade. The spots usually increase rapidly in size, soon become gray at the center, and have an irregular but sharply defined margin. On the most susceptible hybrids large areas of the leaf blade become invaded by the fungus. On the lower surface, the leaf blade and particularly the veins in the affected area become dark brown with white sporodochia along the veins. On the upper surface, small white masses of conidia soon appear, usually developing in concentric circles (FIG. 1, *A*). Along

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[1] Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture, in cooperation with Yale University, New Haven, Conn., and the Northeastern Forest Experiment Station, Upper Darby, Pa. The earliest observations of the disease were made by Dr. E. J. Schreiner of the Northeastern Forest Experiment Station, who instigated the study of the causal organism. The cultural and pathological phases of the study were carried on by the senior author, assisted by Kenneth F. Aldrich, Biological Science Aid in the same Division.

[2] Associate Mycologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland. The junior author identified the causal organism and prepared the description of the species.

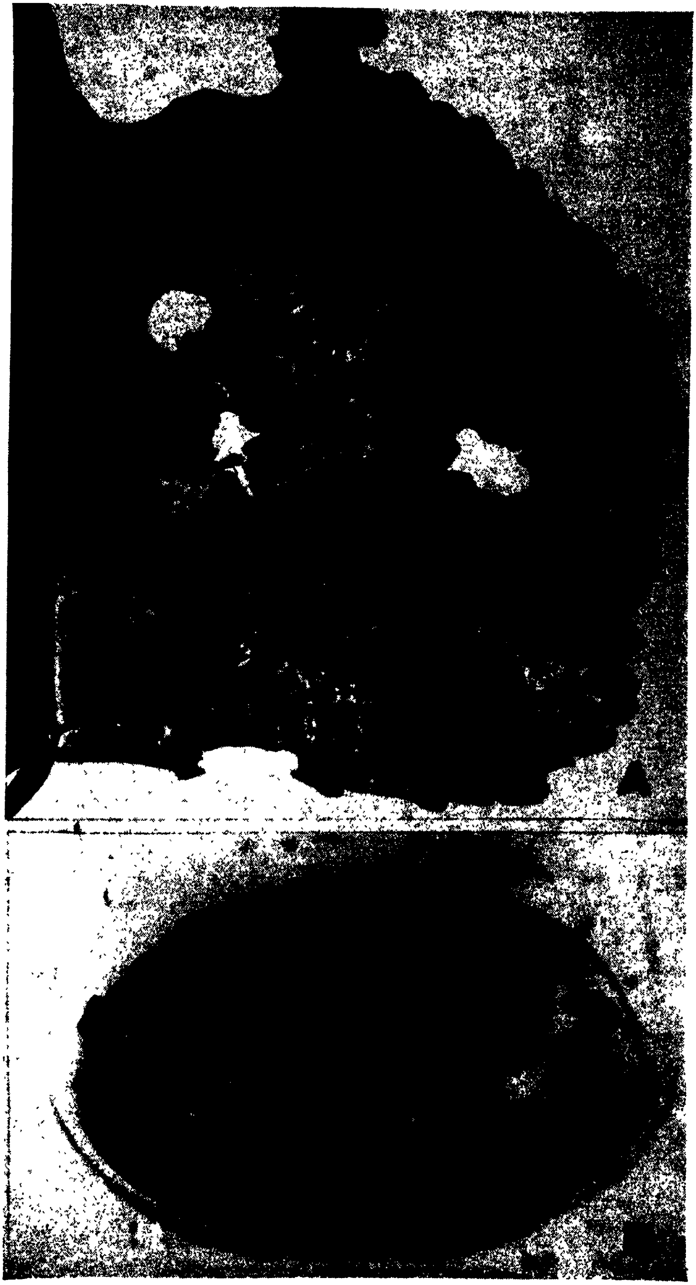

FIG. 1. *Septotinia populiperda.*

the margin of the blotch, white fans of radiating hyphae are frequently formed just below the cuticle of the upper leaf surface, and these, together with a zonate appearance of the entire blotch as the leaf tissue becomes affected, are conspicuous characteristics of the disease. Individual spots may reach a diameter of 1.5 to 2 inches, and two or more of these spots may coalesce, resulting in almost complete invasion of the leaf tissue. Early defoliation may follow and the young stems of the most susceptible hybrid poplars may thus be entirely defoliated by late summer.

IDENTITY OF THE FUNGUS

The fungus present on the leaf blotches produces an abundance of hyaline conidia borne on branched conidiophores which are compactly aggregated to form sporodochia. The conidia are ellipsoid and continuous at first, later becoming 1–3-septate, constricted and frequently breaking apart into two-celled fragments. On examination of the literature it was found that this fungus had been described in 1932 in a paper by Moesz (3, p. 41–42, FIG. 5) as occurring on *Populus deltoides* Marsh. in Latvia, under the name of *Septogloeum populiperdum* Moesz & Smarods. A collection on the same host, made in 1930, was also listed by Smarods (4, p. 50) without description. Two specimens of *S. populiperdum* on *Populus canadensis* Moench from Latvia deposited in the Mycological Collections of the Bureau of Plant Industry have been examined, and the fungus was found to be identical both in macroscopic and microscopic characters with that found on the poplar hybrids in the United States. No references to *S. populiperdum* other than the two cited have been found. Neither of these articles contains a discussion of the distribution or economic importance of the leaf blotch, but the specific name would indicate the destructiveness of the fungus.

Septogloeum populiperdum differs from typical species of *Septogloeum* in several respects. Its closest affinities are with the conidial stage of *Septotinia podophyllina* which was described as *Gloeosporium,* and later referred to *Septogloeum.* Whetzel (5) pointed out that the conidial stage of the fungus on *Podophyllum* is not referable to any described genus but felt it unnecessary to give it a name since the perfect stage is known.[3] The apothecial stage of the poplar fungus, although not yet found in nature, has been developed in culture and found to agree with that of *Septotinia.* Since the spermatial and sclerotial stages characteristic of *Septotinia* have also been found in the fungus on poplar, it is therefore described as the second species of that genus.

Septotinia populiperda Waterman & Cash, sp. nov.

Conidial stage: **Septotis populiperda** (Moesz & Smarods) Waterman & Cash, comb. n. (Syn. *Septogloeum populiperdum* Moesz & Smarods, Magyar Bot. Lapok **31**: 41. 1932).

Sporodochiis amphigenis, saepius epiphyllis, albo-pulverulentis, 80–500 μ in diam.; conidiophoris dense compactis, hyalinis, septatis, 20-24 × 6-12 μ, cellula apicali interdum triangulatim e bullis pluribus ornata, 12–15 μ lata; conidiis hyalinis, unicellularibus vel 1-3-septatis, basibus truncatis, apicibus acutis, ad septa constrictis et dirumpentibus, 15–45 × 4-7 μ; *sclerotiis* per nervos foliorum dispositis, atris, angularibus, elongatis vel circularibus, applanatis, 3–5 × 1-2 mm., interdum coalescentibus; *spermadochiis* e spermatiophoris paucis vel numerosis et dense compactis, verticillatim ramosis, e stirpe centrali oriundis, prope apices compressis dein in forma collaris inflatis compositis; spermatiis globosis, nucleatis, hyalinis, 1.5-2 μ in diam., in catenulis vel massis agglutinatis; *apotheciis* caespitosis, lento-carnosulis, longe stipitatis, breve cupuliformibus, centro plicato depressoque et margine revoluto, hymenio avellaneo, 2-7 mm. in diam., 1-2 mm. altis, stipibus tenuibus, tortis, 1-2 cm. longis, 1 mm. crassis, brunneis; ascis cylindricis, apicibus crasse tunicatis et obtusis, breve pedicellatis, octosporis, 125–135 × 6.6–8.5 μ; ascosporis hyalinis, ovoideis, glabris, aliquantum inaequilateralibus, 10.2–13.2 × 4–5.5 μ; paraphysibus hyalinis, numerosis, flexuosis, ramosis, apicibus ad 1.5 μ inflatis; strato subhymeniali flavidulo, plectenchymatico, exteriori hyalino, pseudoparenchymatico, cellulis 30–40 μ in diam., subglobosis, tenuitunicatis composito.

Sporodochia amphigenous, usually epiphyllous, developing from hyaline subcuticular mycelium on large brownish-gray spots, white, pulverulent, 80–500 μ in diam.; conidiophores densely compact, hyaline, septate, thick-walled, slender below, becoming thicker toward the apex, 20–40 × 6–12 μ, the apical cell sometimes irregularly lobed or triangular, 12–15 μ wide, with 2–3 knoblike protuberances; conidia hyaline, at first unicellular, oblong or acute at one end, 12–15 × 6–9 μ, later 1–3-septate, obtuse at the base, acute at the apex, constricted at the central septum and breaking into 2-celled sections before germination, 2-celled conidia 15–25 × 4–7 μ, 3–4-celled conidia 30–45 × 4–7 μ. *Sclerotia* formed along leaf veins, black, angular, elongate, or circular, flat, 3–5 × 1–2 mm., sometimes coalescing. *Spermadochia* composed of loose to compact groups of verticillately branched hyaline spermatiophores arising from a central stalk, compressed near the apices, then sometimes inflated in the form of a collar; spermatia globose, 1-celled, hyaline, 1.5–2 μ in diameter, each with a large nucleus, often adhering in chains or masses. *Apothecia*[4] single or more often caespitose, tough fleshy, long stipitate, shallow cupshaped, with depressed slightly plicate center and revolute margin, hymenium avellaneous[5] to light cinnamon drab, exterior cinnamon drab to wood brown, 2–7 mm. in diameter, 1–2 mm. deep; stipes long, slender, contorted and spirally twisted, 1–2 cm. long, 1 mm. thick, wood brown becoming bone brown in age; asci cylindrical, wall thickened and somewhat flattened at the apex, slightly narrowed to a short pedicel, 8-spored, 125–135 × 6.6–8.5 μ; ascospores hyaline, ovoid, smooth, unsymmetrical, swollen on one side and depressed on the other, 10.2–13.2 × 4–5 μ; paraphyses numerous, flexuous, hyaline, branched about 20 μ below the apices, 1–15 μ thick; subhymenial layer pale yellowish, plectenchymatic, outer layer hyaline, pseudoparenchymatic, composed of subglobose, hyaline, thin-walled cells 30–40 μ in diameter.

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[3] Buchwald (Contr. Dept. Pl. Path. R. Veter. & Agr. Coll. Copenhagen No. 32, p. 104, 1949) has recently proposed the generic name *Septotis* for the conidial stage of *Septotinia.*

On leaves of hybrid clones of *Populus* spp., Maine, Vermont, Connecticut, Massachusetts, New York, Pennsylvania, and Maryland; also on leaves of *P. trichocarpa* Hook. and *P. fremontii* S. Wats., growing in the vicinity of infected hybrid poplars in Maryland.

Apothecia produced in type culture derived from conidia on leaves of clone of hybrid (*P. deltoides virginiana* (Castiglioni) Sudw. × *P. trichocarpa*), FP97101, are deposited in the Mycological Collections of the Bureau of Plant Industry, Beltsville, Md.

Specimens examined: *Septogloeum populiperdum* Moesz & Smarods on *Populus canadensis* Moench. Salaspils, Prov. Vidzeme, Latvia, Sept. 25, 1932, J. Smarods, Fungi latvici exsiccati No. 241; Stopini, Prov. Vidzeme, Latvia, July 20, 1935, coll. J. Smarods, ex Herb. J. Smarods.

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[4] Description of apothecia is based on specimens grown in culture.

[5] Color readings are from Ridgway, R., Color standards and color nomenclature. Washington, 1912.

Although very similar to *Septotinia podophyllina* in all of its stages, the species on poplar shows some morphological differences, such as larger apothecia, contorted and spirally twisted stipes, and smaller, unsymmetrical ascospores. The spermatia are also smaller and globose instead of ovate, and the 1- and 2-celled conidia are shorter than those in *S. podophyllina.*

CULTURAL CHARACTERISTICS

Ten monoconidial isolates from leaf blotches on 8 clones of 6 hybrid poplars were cultured for a comparative study of variations among the isolates. Conidia, usually two-celled before germination, produced germ tubes readily from both cells in hanging-drop water culture or on potato-dextrose agar within 18 hours. A vigorous growth of white, fluffy, aerial mycelium developed on both malt extract and potato-dextrose agar. At room temperature colonies developed to about 25 mm. in diameter in a week and spermadochia with abundant spermatia were produced. In monoconidial cultures on potato-dextrose agar in 150 cc. Erlenmeyer flasks, the advancing edge of the colony soon showed a fanlike growth of radiating strands of hyphae similar to that occurring at the margin of a leaf blotch. The aerial hyphae gradually became more appressed to the agar surface, forming a thick mycelial mat. In some monosporous colonies the mycelium remained white, but in others it became tan or yellowish brown, gradually darkening to gray brown. In about 10 days scattered compact masses of mycelium appeared and these gradually darkened, forming black sclerotia (FIG. 1, *B*). In all isolates the sclerotia were circular at first, and in the flask cultures were distributed in concentric circles. The isolates from the various leaf blotches differed considerably in the ultimate size and shape of the sclerotia. Some of these remained circular while others were irregular and later tended to coalesce, forming a thick crust, frequently covered with a grayish-brown mycelial mat. However, the conidia from an individual leaf blotch developed colonies which resembled one another very closely. Sclerotia were abundant in the isolates from some blotches, but were rare in those from others. In the latter, amber-colored gelatinous masses of spermatia were conspicuous on the spermadochia which usually were arranged in concentric circles (FIG. 2, *A*). This variation in production of sclerotia and spermatia suggests the plus and minus strains reported by Whetzel for *Septotinia podophyllina* (**5**).

In some of the flask cultures on potato-dextrose agar at room temperature, receptive bodies similar to those described by Drayton for *Sclerotinia gladioli* (**2**) began to appear about a month after the sclerotia had formed. The receptive bodies (FIG. 2, *B*), which developed singly or in groups directly from the sclerotia, were very slender columns 10–20 mm. long, slightly hairy, cream- or fawn-colored, tapering to a darker apex. The columns became curved or twisted as they lengthened, apparently in response to light stimulus. In agar cultures these bodies failed to develop further and soon withered. Spermatization was attempted by growing two isolates in proximity in a flask and also by pouring over the sclerotia a water suspension of spermatia from another isolate. The receptive bodies, however, showed no response to either treatment. Therefore, a method of spermatization based on that devised by Drayton (**2**) was developed. Entire sclerotial layers from the potato-dextrose agar cultures with receptive bodies were transferred to flasks containing a three-fourth inch layer of moist sterilized sand. A water suspension of spermatia from another isolate was poured over the sclerotia and also applied to the tips of the receptive bodies by means of a sterilized camel's-hair brush. A thin layer of a moist sterilized mixture of soil and leaf mold was then sprinkled over the surface of the sclerotia. No further development of the receptive bodies took place. In a second attempt at spermatization a water suspension containing a mixture of spermatia from four isolates was similarly applied to sclerotia and receptive bodies in sand cultures, and a thin surface layer of the moist sterilized soil was added. Within a week at room temperature the tips of the receptive bodies began to darken slightly and expand, eventually developing into fawn-colored apothecia (FIG. 2, *B*, *C*) with mature ascospores. It was found that receptive bodies appeared more rapidly when the sclerotial layer was transferred from potato-dextrose agar to moist sand as soon as it was well developed. By this method the entire process of development from the isolation of a single conidium to the maturing of the ascospores required about 10 to 12 weeks at room temperature.

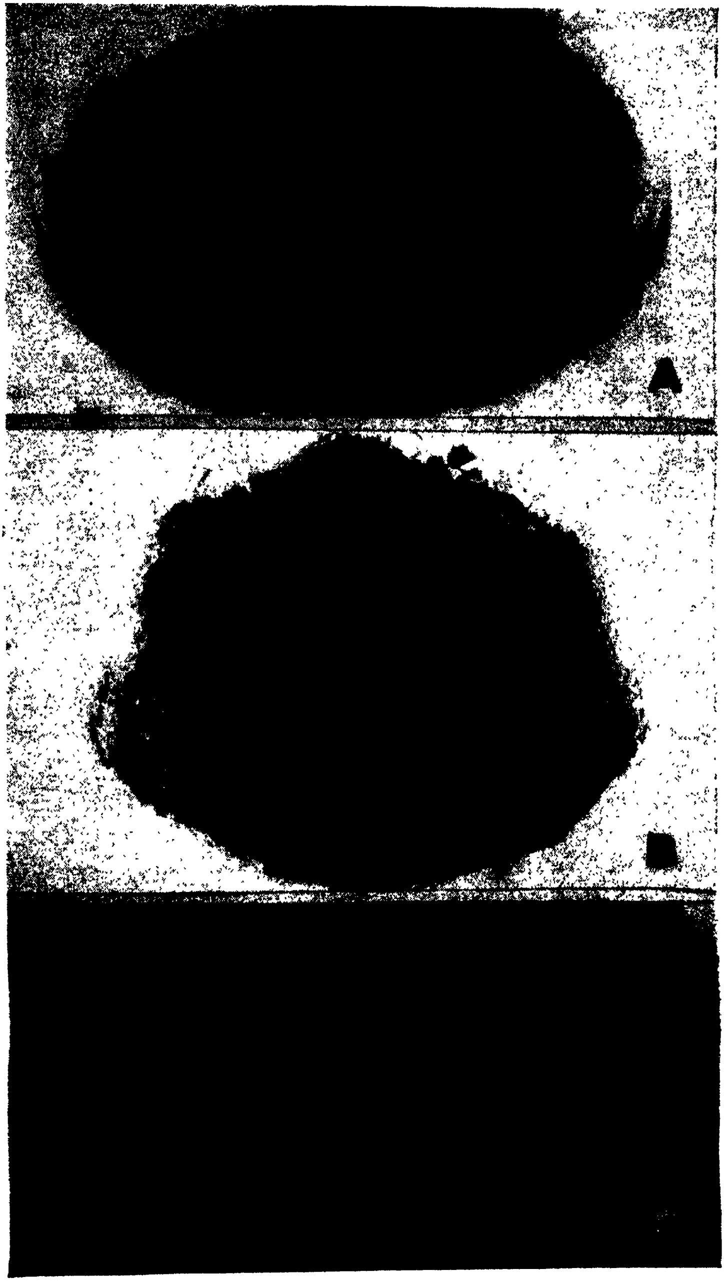

FIG. 2. *Septotinia populiperda.*

Of the ten isolates used in this study only four developed receptive bodies and mature apothecia. In these four, the sclerotia were abundant but spermatia were comparatively rare. The remaining isolates formed an abundance of spermatia, but the sclerotia were mostly limited to the outermost portion of the culture next to the glass of the flask.

No conidia have been produced in culture. The spermadochia, spermatia, apothecia, and ascospores resembled very closely those described and illustrated by Whetzel for *Septotinia podophyllina* (5). However, no study of temperature relations in the growth of the poplar fungus has been made, and color reactions in the agar, reported by Whetzel, have not been observed.

The spermadochia showed considerable variation in development and form. In some cultures they were composed of a short central stalk with a rather loose group of verticillately branched spermatiophores, from the tips of which the spermatia were produced in basipetal succession. The spermatia were held together in gelatinous masses but the general appearance of the mycelial surface of the culture was diffuse and powdery. In other cultures the spermadochia were large compact groups of verticillately branched spermatiophores arising from a stout central stalk which usually was composed of a strand of closely woven hyphae. The spermatia developed in abundance on the tips of the spermatiophores, forming dense gelatinous masses. These masses of spermatia gave an amber-colored waxy appearance to the surface of the culture.

Apothecia have not yet been collected in nature but spermadochia have been found on the blades of fallen leaves and smooth black sclerotia on the veins. Cultures from these sclerotia have produced characteristic mycelial growth, with spermatia and sclerotia.

PATHOGENICITY

The leaves of small potted poplar trees developed from cuttings and growing in the greenhouse were inoculated with a suspension of ascospores. The solution used for the suspension was the sodium-oleate-gelatin mixture devised by Andersen and Henry (1), and made according to the formula which proved to be the most effective spreader-sticker combination in their experiments. Tests with the *Septotinia* ascospores in the mixture in hanging-drop cultures showed a high percentage of germination in 24 hours.

The species and hybrids of poplars tested were as follows: 2 clones of *Populus fremontii*, 2 clones of *P. trichocarpa*, 2 clones of *P. charkowiensis* Schroed. × *P. trichocarpa*, 1 clone of *P. sargentii* Dode × *P. berolinensis rossica*, 1 clone of *P. petrowskyana* Schneid. × *P. caudina*, 1 clone of *P. candicans* Ait. × *P. berolinensis* Dipp. One tree of each clone, averaging about 14 leaves per tree, was inoculated by placing droplets of the ascospore suspension on both surfaces of each leaf by means of a pipette, allowing the droplets to spread naturally. Satisfactory spread of the suspension was obtained on both leaf surfaces. One additional tree of each of the hybrid clones and one of *P. fremontii* were similarly treated with the sodium-oleate-gelatin mixture without spores and were maintained as controls. All trees were placed under a humidity tent on the greenhouse bench for 48 hours.

Within two weeks small spots appeared on a few of the leaves of the 2 clones of the *charkowiensis* × *trichocarpa* hybrid. The spots developed rapidly and sporodochia with mature conidia were produced. Four weeks after inoculation monoconidial isolations were made on potato-dextrose agar and the resulting cultures developed characteristic spermatia and sclerotia. Leaves of the other hybrid clones failed to develop leaf spots. On the tree of one clone of *P. trichocarpa* a few leaves showed a browning of the midrib and veins suggestive of that occurring on infected leaves under natural conditions but no leaf blotches or sporodochia developed and leaf cultures failed to produce characteristic *Septotinia* mycelium. No indication of infection was evident on the leaves of the trees used as controls.

These limited results, together with field observations, indicate the pathogenicity of the fungus, and the rapid development of the leaf blotch following infection. The conditions favoring the spread of the disease in plantings of hybrid poplar clones and the relative susceptibility or resistance of native and introduced poplar species as well as clones of the hybrid poplars are being studied.

SUMMARY

A leaf blotch on young plantation trees of hybrid poplar clones in the eastern United States is caused by a hitherto unknown spe-

cies of *Septotinia,* described as *S. populiperda.* Large areas of the leaf blades may become affected and extensive premature defoliation may result. Sporodochia with white masses of conidia are found on both surfaces of the leaf spots, usually developing in concentric circles on the upper surface.

Spermadochia with verticillately branched spermatiophores bearing masses of spermatia form on fallen leaves, and small black sclerotia may be found along the veins. Apothecia have not been observed in nature but were produced in pure culture on sterilized sand, following the spermatization of receptive bodies with compatible spermatia.

The leaves of trees of five hybrid poplar clones and of two species of poplar were inoculated in the greenhouse with a suspension of ascospores. Typical leaf blotches with sporodochia and conidia were produced on two clones of the hybrid *Populus charkowiensis* × *P. trichocarpa.*

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EXPLANATION OF FIGURES

FIG. 1. *A.* Upper surface of portion of poplar leaf with blotches, showing white masses of conidia in concentric circles and white fans of radiating hyphae at margins of blotches; natural size. Photograph by R. L. Taylor. *B.* Sclerotia in monoconidial culture on potato-dextrose agar; natural size. Photograph by H. G. Eno.

FIG. 2. *A.* Spermadochia in concentric circles on potato-dextrose agar. *B.* Apothecia developed from cross-spermatization of receptive bodies in flask culture, on sterilized sand, soil, and leaf mold; receptive bodies in the background; natural size. *C.* Mature apothecia in flask culture; three times natural size. Photographs by H. G. Eno.

A NEW GENUS OF THE TREMELLALES FROM LOUISIANA

Lindsay S. Olive

(With 2 Figures)

During a field trip in a swampy wooded area near Baton Rouge, Louisiana, the writer collected a jelly fungus which seems not to have been previously described. The fungus, which clearly belongs in the Auriculariaceae, was found growing on a fallen dead limb of a frondose tree (fig. 1, *1*). Although it was found to resemble *Platygloea* in some ways, there are several important features which prevent its inclusion in that genus, at least without a revision of the present concept of *Platygloea*. The writer considers it advisable to erect a new genus to accommodate this new species of jelly fungus.

Mycogloea gen. nov.

Fructificationibus gelatinosis, indeterminatis; basidiis bacillariis et transverse-septatis, sterigmata comparative brevia gerentibus; basidiis gemnatis ex cellis binucleatis persistentibus in modo structuarum vacuarum basibus basidiorum, e quibus facile separabiles sunt.

Type species *M. carnosa.*

Fructifications gelatinous, indeterminate; basidia rod-like, transversely septate, producing relatively short sterigmata, budded out by binucleate cells, which persist as empty structures at their bases and from which they readily become detached.

Mycogloea carnosa sp. nov.

Fructificationibus firmiter gelatinosis, colorem carnosum typice habentibus. Pustulibus, 0.4–2.4 mm. diam., coalescentibus et usque ad 12 mm. longis attinentibus. Hymenio consistente paraphyses, conidiophora, et basidia. Paraphysibus curvatis vel circinatis terminalibus, 1.7–2.5 μ diam., conidiis ovatis vel elongatis, 2.7–3.8 × 3.8–9.1 μ. Basidiis 3.2–4.9 × 21–50 μ, angustis, cylindraceis quattuor cellulas habentibus, gemnatis ex binucleatis cellulis subbasidiis, quibus facile separatis sunt, germinantibus et productentibus sporidia vel basidiosporas. Sporidiis 2.4–3.8 × 3.8–6.1μ; basidiosporis apiculatis, 2.7–3.4 × 6.1–8.4 μ. Haec species in frondoso ramo demortuo crescit, Fructifications firmly gelatinous or cartilaginous-gelatinous, sordid white to light orange, typically flesh-colored, at first appearing as small pustules 0.4–2.4 mm. in diameter, pustules coalescing and forming flattened tuberculate masses up to 12 mm. or more in length, indeterminate. Drying to thin, horny, amber-colored to dingy brown masses. Hymenium containing paraphyses, conidiophores, and basidia. Paraphyses sparingly branched, curved, or circinate at their tips, 1.7–2.5 μ in diameter; conidiophores irregularly branched, producing oval to elongate conidia measuring 2.7–3.8 × 3.8–9.1 μ; basidia straight, narrow, 4-celled, 3.2–4.9 × 21–50 μ, produced by binucleate sub-basidial cells from which they are easily detached at maturity, germinating and producing basidiospores on short sterigmata, or giving rise to an indefinite number of conidia; basidiospores apiculate, flattened on one side, 2.7–3.4 × 6.1–8.4 μ; basidial conidia mostly oval, 2.4–3.8 × 3.8–6.1 μ.

A single collection was made on a dead corticate frondose limb, near Baton Rouge, Louisiana, April 28, 1949.

Microscopic examination of the fungus reveals the presence of a hymenium containing numerous paraphyses, which are characteristically curved or coiled at their tips (FIG. 1, *2*). The ends of the paraphyses often undergo gelatinization, a process which can be hastened by the addition of potassium hydroxide solution to the mount. The straight, rod-like basidia arise in proliferating clusters apically and on lateral branches of fertile hyphae with clamp connections, or on lateral branches which originate at different intervals along the lower portions of the paraphyses. The paraphyses extend considerably beyond the basidia. Also present in the hymenium, along with the basidia and paraphyses, are hyphae bearing conidia. These hyphae are irregularly branched, and no clamp connections were found on them (FIG. 2, *5*). Conidia are produced in large numbers by these hyphae.

When a fresh mount of the mature fructification is made for microscopic observation, one of the most impressive features is the abundance of detached basidia floating about in the water, along with a large number of conidia (FIG. 1, *2*). The basidia apparently become readily detached in nature, many of them rising to the surface of the fructification. It is difficult to find germinating basidia with basidiospores still attached, and most of the basidia seem to bud out indefinite numbers of conidia in a manner characteristic of the basidia of many smut fungi. If basidia are spread out on plain agar to germinate, the conidia are produced even more profusely (FIG. 2, *1–4*). These conidia are capable of budding in a yeast-like manner. When a mass of basidia is placed in a drop of water on a slide and kept in a moist chamber for a while, most of the basidia produce conidia upon germination. However, many of the basidia located near the surface of the water produce basidiospores (FIG. 1, *4*; 2, *20, 21*). These spores are produced above the surface of the water. It seems likely, therefore, that in nature the basidia which remain farther down in the fructification produce conidia; whereas, those which drift up closest to the surface after becoming detached

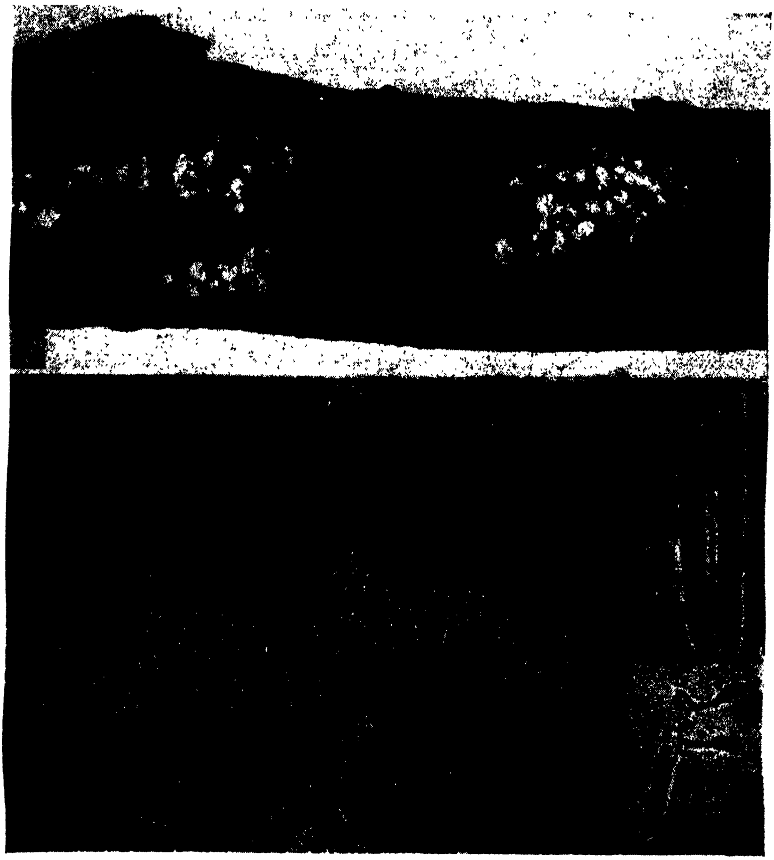

FIG. 1. *Mycogloea carnosa.*

are more likely to produce basidiospores. The latter produce their basidiospores on relatively short sterigmata and resemble the basidia of a typical rust fungus.

With the use of propiono-carmine smear preparations of the hymenium, the writer has been able to make a brief cytological study of the organism. Nuclear details in the conidiophores were not observed, but the conidia were found to be both uninucleate and binucleate (FIG. 2, *6*): The fertile hyphae are dikaryotic, with clamp connections at the septa (FIG. 2, *7*). The basidia are consistently produced by special binucleate cells which proliferate, with the aid of clamp connections, from the ends of fertile branches. Each basidium is produced in the following manner. A terminal binucleate cell with a clamp connection at its base produces a bud at its tip (FIG. 2, *8*). The two nuclei and some cytoplasm migrate into the bud cell (FIG. 2, *9*). Both the bud cell and the cell which produces it continue to elongate, and the two nuclei in the bud cell fuse (FIG. 2, *10–12*). It is obvious, therefore, that the young bud cell is a probasidium. The cell beneath it is referred to by the writer as a sub-basidial cell. The fusion nucleus now divides and a septum appears across the middle of the basidium dividing it into two uninucleate cells (FIG. 2, *13, 14*). A second nuclear division occurs and a 4-celled, 4-nucleate basidium is produced (FIG. 1, *3*; 2, *15, 16*). These two successive nuclear divisions, in all likelihood, are meiotic. By this time the sub-basidial cell is much elongated, devoid of cytoplasm, and somewhat collapsed (FIG. 2, *17*). The mature basidium easily becomes detached from the empty sub-basidial cell.

When the basidium produces basidiospores, each cell gives rise to a sterigma which, in turn, produces a single uninucleate basidiospore at its tip (FIG. 2, *18–21*). The basidium is entirely emptied of protoplasm as the four spores are produced. On the other hand, when the basidium produces conidia, several conidia may bud out either directly or on short protrusions from each cell. The basidial nuclei divide and produce the conidial nuclei, and the basidium retains its 4-nucleate condition; the basidial conidia are uninucleate (FIG. 2, *22*).

According to Martin (Univ. Iowa Stud. Nat. Hist. **18**: 67.

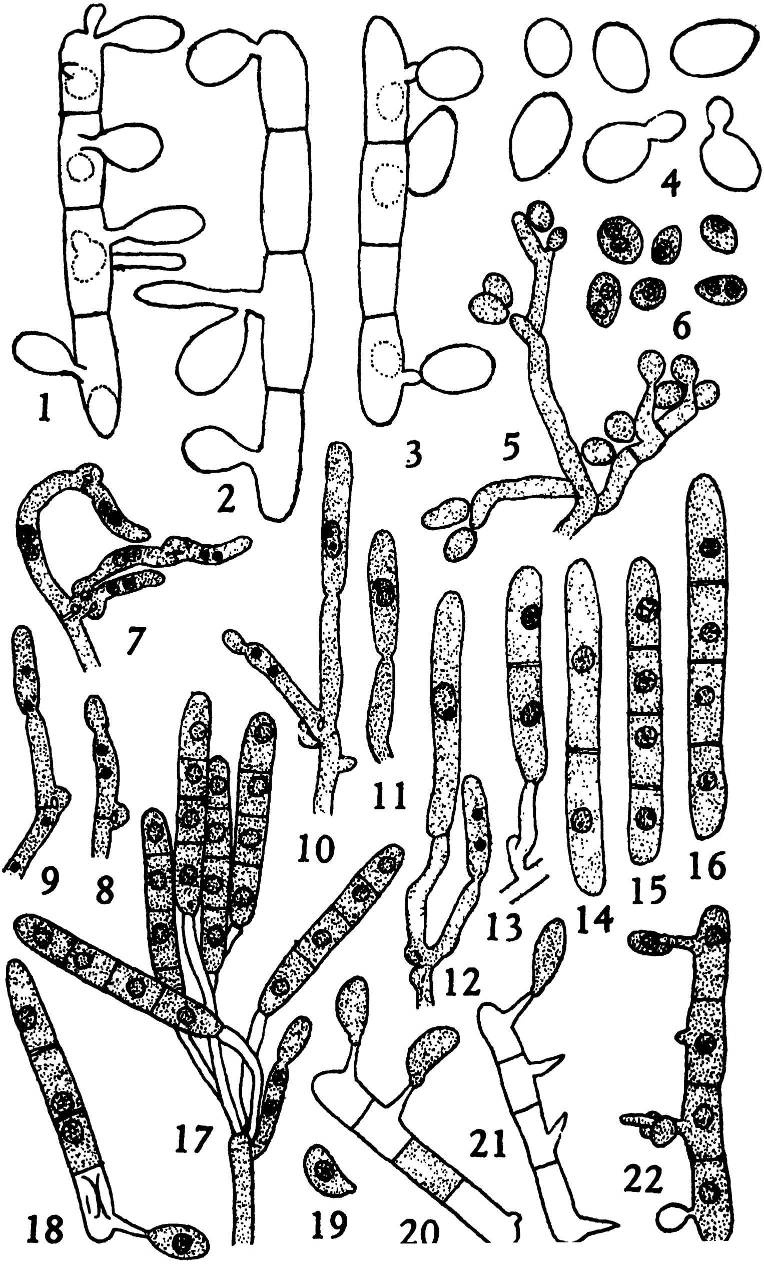

FIG. 2. *Mycogloea carnosa.*

1944), at least one species of *Platygloea,* namely *P. fimicola* Schroet., may have persistent probasidium-like structures ("hypobasidia"). There is no information concerning cytological developments in that species, however. Furthermore, there might be some question as to whether a species with persistent probasidia should be included in *Platygloea.* In any case, the present fungus differs markedly from *P. fimicola* and from all other species of *Platygloea* known to the writer in that it produces straight, rod-like, easily detachable basidia, which may germinate normally to produce indefinite numbers of conidia, or which may produce basidiospores on relatively-short awl-like sterigmata. These are unlike the sterigmata of *Platygloea,* which typically become considerably elongated. The sub-basidial cells are also a conspicuous feature of the new fungus. Although they may superficially resemble persistent probasidial cells at the bases of the mature basidia, they are not considered to be true probasidia, since nuclear fusion does not occur in them, but in the cells budded out by them.

The writer is grateful to Dr. C. W. Edgerton, Department of Botany, Louisiana State University, for his assistance in the preparation of the photographs, and to Dr. Concetta C. Bellini for her preparation of the Latin descriptions.

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EXPLANATION OF FIGURES

FIG. 1. *1–4.* Photomicrographs. *1,* Fructifications on dead wood (× 1.5); *2,* crushed preparation of hymenium, showing curved paraphyses, basidia, and conidia (× 240); *3,* two 4-celled, 4-nucleate basidia and conidia (× 1400); *4,* basidium producing basidiospores (× 780).

FIG. 2. *1–22.* Microscopic study of living and stained material (all figures × 1380). *1–3,* basidia giving rise to conidia on agar; *4,* basidial conidia, some budding; *5,* conidiophores producing conidia; *6,* conidia, uninucleate and binucleate; *7,* hyphae with clamp connections and binucleate cells; *8, 9,* binucleate cells budding out basidia; *10, 11,* nuclear fusion in young basidia; *12,* young basidium with fusion nucleus; binucleate probasidium; *13, 14,* two-celled basidia after first meiotic division; *15, 16,* mature 4-celled, 4-nucleate basidia (detached) after second meiotic division; *17,* cluster of mature basidia still attached to empty sub-basidial cells; *18–21,* basidia germinating and producing uninucleate basidiospores; *22,* basidium budding out uninucleate conidia.

A NEW ACHLYA FROM MACKINAC ISLAND, MICHIGAN, WITH NOTES ON OTHER SPECIES[1]

T. W. Johnson, Jr.[2]

(WITH 2 FIGURES)

Several studies relating, at least in part, to the presence of saprolegniaceous fungi in the soils and waters of various localities in Michigan have been published in the past by such workers as Kauffman, Pieters, and Kanouse. The region north of the University of Michigan Biological Station, near Cheboygan, Michigan, has not, however, been the object of any collections made specifically for the Saprolegniaceae, either by these or other investigators. In particular, Mackinac Island has, it would appear, been overlooked as a possible source for these fungi.

Opportunity was afforded on July 18, 1948, of obtaining from the Island, collections of soil and water which might yield saprolegniaceous fungi. The results of this preliminary survey were promising indeed, since, out of a total of 61 collections, 49 isolates were obtained, representing 4 genera, 9 previously described species and one variety, 6 non-fruiting isolates, and one apparently new species. Five of the known isolates, *Achlya Rodrigueziana* F. T. Wolf, *A. flagellata* Coker, *Saprolegnia delica* Coker, *S. monoica* var. *glomerata* ? Tiesenhausen, and *Dictyuchus anomalus* Nagai, have not, heretofore, been reported from Michigan. Figure 1 shows the areas in which collections were made.

Samples were taken from seventeen areas on Mackinac Island. The collections were made from such diverse localities as: water

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[1] Contribution No. 898 from the Department of Botany, University of Michigan.

[2] The author wishes to express his deepest appreciation to Professor F. K. Sparrow, for his helpful suggestions and criticisms in the preparation of this paper, and to Mrs. T. W. Johnson, for assistance in preparation of the figures.

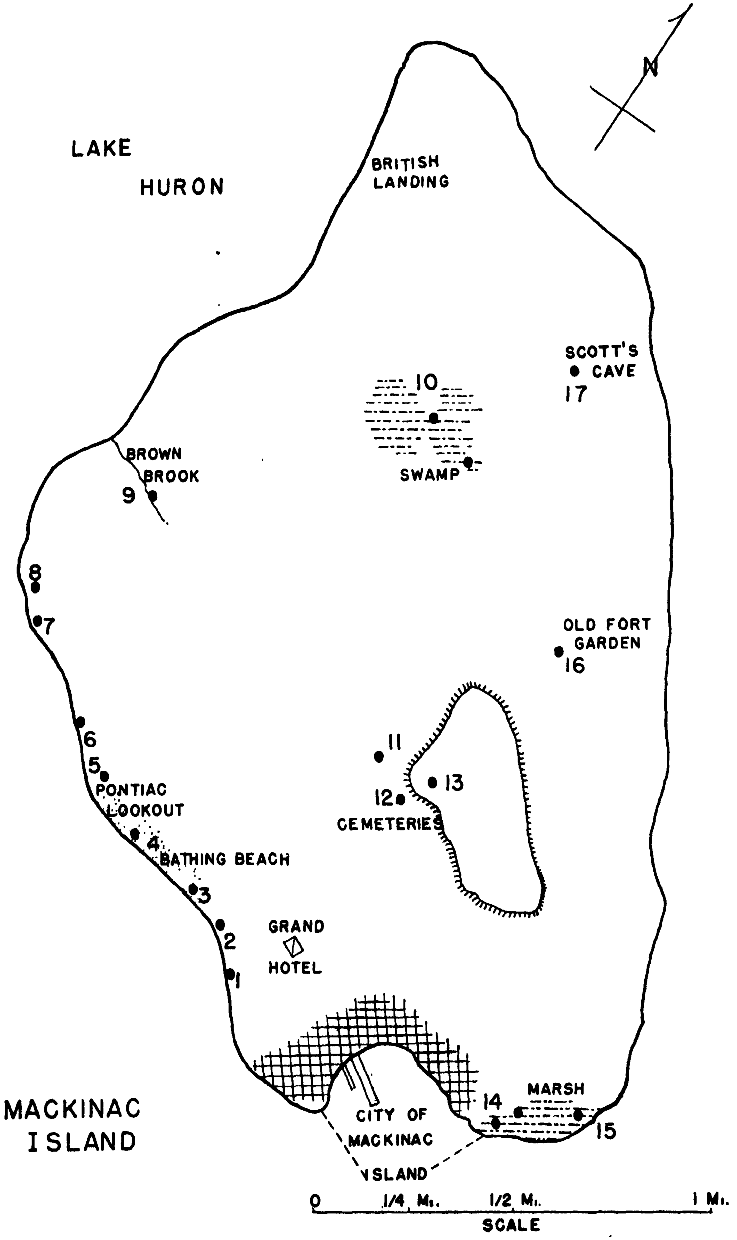

FIG. 1. Sketch of Mackinac Island, Michigan, showing areas of collection and points of orientation. Scale: 1″ = appx. 800 yards.

from beach pools and inland pools; sandy, dry soil from the margins of beach pools and near the vegetation line; humus from the forest floor; muck from an intermittent bog; and soils and water from marsh areas adjacent to the beach line. An abundance of dead, young lake trout (*Cristovomer* sp.) bearing a cottony mycelial growth were found in a few of the beach pools. Three of these fish were taken as part of the collections.

The samples were subjected to the usual well-known methods of obtaining these fungi. Single spore isolates were made of all species as they were recovered from the samples. All identifications and descriptions are based on colonies 12-16 days old, growing on half of a boiled hempseed, in 30 cc. of sterile, charcoal-filtered, distilled water, at a temperature of 22 ± .4° C. Percentages, averages, *etc.*, are reported on the basis of 500–700 observations or measurements of the morphological features concerned.

Three collections from the Island yielded a fungus, intermediate between *Achlya Klebsiana* Pieters and *A. Rodrigueziana* F. T. Wolf. The plant, however, is sufficiently distinct from these and other known species to be considered as new.

Achlya michiganensis sp. nov.[3] FIG. 2

Myceliis in semine *Cannabis sativae* densis, quandoque porrectis usque ad 1–1.5 cm. in diametrum; hyphis primariis in basi 40–50 μ diam. Sporangiis copiosis, cylindratis aut attenuatis, inter 152-210 μ longis et 17–22 μ diam.; cymosis, zoosporiis 9.9–11.0 μ diam., germinantibus sicut in Achlyis; aliquibus secondariis sporangiis excrescentibus ex 1–3 lateralibus papillis. Gemmis paucis, variis, plerumque inaequaliter formatis. Oogoniis copiosis et globosis; natis ex primariis hyphis in ramulis lateralibus, curvis aut raro rectis, nonnumquam semel usque ad quater aut magis pro diametro cuiusque oogonii, sed plerumque bis usque ad ter; raro terminalibus, numquam intercalaribus; 35–66 μ diam., plerumque 38.5–49.5 μ; proliferentibus et prolem crescentibus abundanter; tunica levi et tenui, notata nullis punctulis nisi ubi antheridia tangunt. Oosporiis globosis aut ellipticis; excentricis; inter 18.7–28.6 μ diam., sed plerumque inter 22.0–25.3 μ; numero 1–6, plerumque 3, rarissime 8–10. Antheridiis frequentissime dicliniis nonnumquam irregularibus et contortis; persistentibus; antheridio digitalibus prominentiis affixo, 1-4 pro quoque oogonio.

Hab. ad terram humosam, Mackinac Island, Michigan, July 18, 1948.

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[3] Grateful acknowledgment for the preparation of the Latin diagnosis is extended to Professor Bruno Meinecke, Department of Classical Studies, University of Michigan.

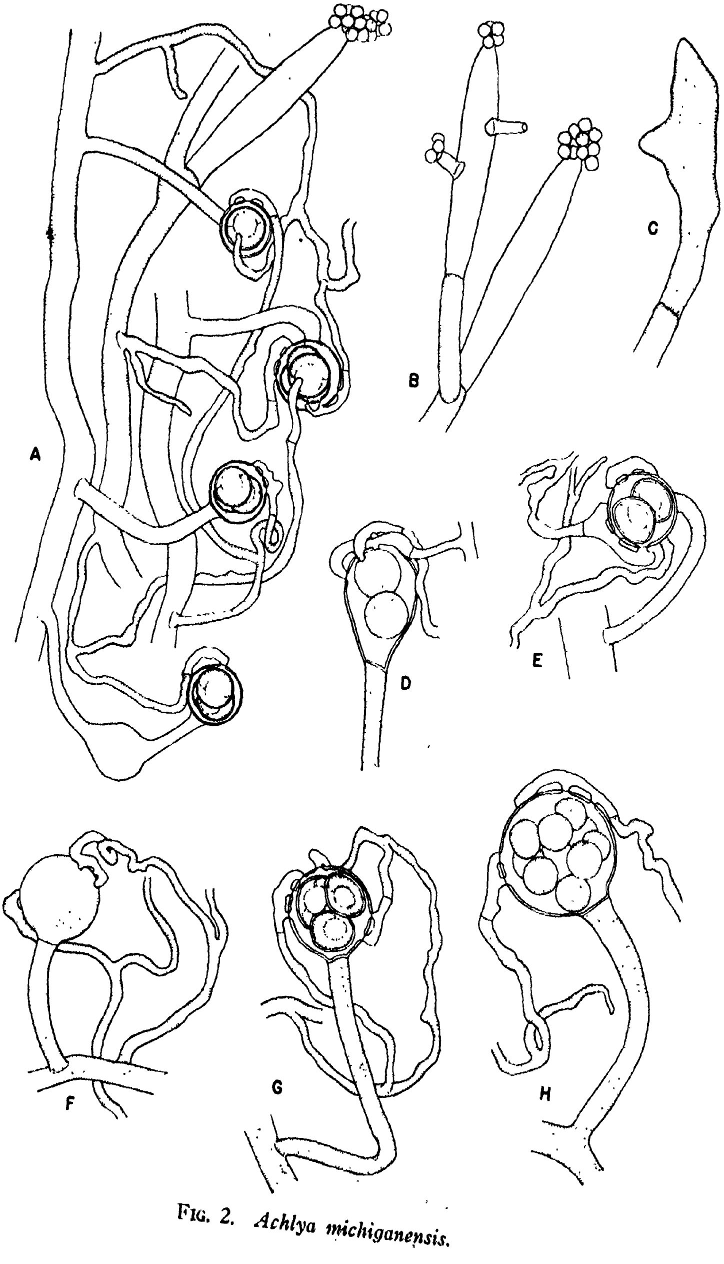

Fig. 2. *Achlya michiganensis.*

Growth on hempseed dense, the colony averaging 1–1.5 cm. in diameter; principal hyphae 40–50 μ in diameter at the base. Gemmae scarce, even in very old cultures; variable in size; irregular; mostly terminal and single, or not in chains of more than two. Sporangia abundant; cylindrical, or tapering toward the apex; predominately broadest near the middle; 112–280 × 14–28 μ, usually 152–210 × 17–22 μ; renewed only by cymose branching; primary zoospores escaping through an apical pore and encysting to form a hollow sphere; in occasional secondary sporangia also escaping through 1–3 long, lateral exit tubes; encysted spores 9.9–11.0 μ in diameter. Oogonia abundant, spherical or short-pyriform; borne laterally along the main hyphae on bent or rarely straight stalks 1–4 or more times longer than the diameter of the oogonium, usually on stalks 2–3 times the diameter of the oogonium; rarely terminal, never intercalary; 35–66 μ in diameter, predominately 38.5–49.5 μ; proliferation of unfertilized oogonia common; wall smooth, thin, unpitted except where antheridial cells are attached. Oospores spherical or ellipsoidal; eccentric, with a single oil droplet not completely surrounded by protoplasm; 18.7–28.6 μ in diameter, predominately 22.0-25.3 μ, averaging 23.7 μ in diameter; in large, abnormal oogonia, predominately 19.0–20.1 μ; 1–6, usually 3 in number, very rarely 8–10[4]; germination not observed. Antheridial branches predominately diclinous; if androgynous, the branch usually originating remote from the oogonium and its stalk; long, much-branched, irregular and contorted; persistent; antheridial cells clearly abstricted, and attached to the oogonium by finger-like projections; 1–4 antheridia to an oogonium; fertilization tubes rarely formed.

From wet humus and sandy soil, Catholic Cemetery, Area 12 (**Type**); dry, sandy soil, near Military Cemetery, Area 13; wet debris and sand from below water line in a pool, Old Fort Garden, Area 16. Mackinac Island, Michigan, July 18, 1948.

The small number of oospores, the small, smooth-walled oogonia which tend to proliferate when unfertilized, the bent or curved oogonial stalks, and the much-branched, irregular antheridial branches, constitute the distinctive features of this fungus.

Achlya michiganensis has been compared with living material of *Achlya Klebsiana, A. Rodrigueziana, A. americana, A. flagellata,* and several of the closely related papillate species of the genus.

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[4] About 5% of the oogonia with 1 oospore, 25% with 2, 40% with 3, 15% with 4, 10% with 5, 5% with 6, and less than .2% with 8 or 10.

The affinity of this Michigan plant to *A. Klebsiana* and *A. Rodrigueziana* is at once obvious.

Achlya michiganensis differs from *A. Klebsiana* as defined by Pieters (8) in the following features: possession of pits in the oogonial walls at the point of attachment of the antheridial cells; formation of longer, bent or recurved oogonial stalks; presence of an occasional androgynous antheridium; frequent proliferation of unfertilized oogonia, and a lesser number of smaller oospores. The living material of *A. Klebsiana* (kindly furnished by Professor J. N. Couch) has characteristics not in complete agreement with the fungus as described by Pieters. This material has, nevertheless, been used as a basis for comparison, although with the reservation that it may not be *A. Klebsiana.* In the final analysis, Pieters' description of *A. Klebsiana* must necessarily be accepted as the correct interpretation. The sporangia which discharge the spores only by an apical pore; the occasional presence of 1–3 long, lateral exit tubes in some secondary sporangia; scarcity of gemmae; more pronounced curve or bend to the longer oogonial stalks; smaller size of the oogonia; the lesser average number of oospores; the much-branched, irregular antheridial branches, and the frequent proliferation of unfertilized oogonia in *A. michiganensis,* are points of distinction between it and the material supplied by Professor Couch.

With respect to oogonial size, eccentricity of oospores, and proliferation of oogonia, there is a resemblance between *A. michiganensis* and *A. Rodrigueziana* (11). On the other hand, the unpitted oogonial walls, smaller number of oospores, fewer androgynous antheridia, parthenogenetic maturation of some unfertilized oogonia, more abundant gemmae, and more extensive growth of *A. Rodrigueziana,* serve to distinguish it from the Mackinac Island isolate.

Proliferation of oogonia in *Achlya proliferoides* and *A. flagellata* is suggestive of *A. michiganensis,* but beyond this similarity, the two known species are obviously distinct from the present fungus. The illustrations of *Achlya Oryzae* Nagai (7), from Japan, are suggestive of *A. michiganensis.* Nagai's species may readily be separated from our plant on the basis of the possession of larger oogonia, pitted oogonial walls (rarely not pitted), larger number of oospores, ramified or clasping antheridial cells, and the more frequent androgynous antheridia. Our species is also decidedly different from any of the European species of *Achlya* thus far described.

Preliminary studies concerning the effect of natural environmental conditions (clear stream water, stagnant pool water, *etc.*) on the distinguishing morphological features of *A. michiganensis,* would seem to indicate that the fungus is consistently stable in its characteristics. The formation of more abundant gemmae was not induced by any of the cultural conditions which were utilized. The effect of low temperatures on the production of papillations on the oogonial walls of *A. michiganensis* was also studied. Temperatures of 10, 15, and 20° C. were ineffective in inducing papillate oogonia.

OTHER FUNGI RECOVERED

Saprolegnia parasitica Coker. This species was obtained from the following localities: dead lake trout, in beach pool south of Grand Hotel, Area 1; beach pool near Pontiac's Lookout, Area 5, and beach pool below Lover's Leap, Area 6. Attempts to induce sex organ formation by cultivation of the isolates on the glucose-peptone medium suggested by Kanouse (**5**), were successful in only one of our isolates. In these colonies the fructifications were comparable to those described by Kanouse for *S. parasitica.* The vegetative and asexual features of the sterile isolates were characteristic of the species.

Saprolegnia ferax (Gruith.) Thuret. Isolated from wet humus and sandy soil taken from the bank of Brown Brook, Area 9. Irregular sporangia; large, pitted oogonia which have a tendency toward clustering, and rarely-formed antheridia characterize this species. Another morphological feature which appears of some value in the identification of *S. ferax* is the formation of cylindrical oogonia in old sporangia. In the first of our isolates, Strain M9-1, this feature is of common occurrence, while in the second isolate, Strain M9-2, it is quite infrequent. The percentage of antheridia has varied considerably in our two strains from culture to culture. Strain M9-1 produced antheridia varying from 1.5–76% in twelve consecutive cultures. Strain M9-2, on the other hand, produced antheridia on .6–11% of the oogonia in the same number of subcultures, grown under similar conditions. The oospores in Strain M9-1 were slightly smaller than those reported for *S. ferax,* while in Strain M9-2 these structures agreed in size with the species as interpreted by Coker and Matthews (2).

Saprolegnia mixta deBary. Collected from sandy soil below Stonecliff, Area 7; water from beach pool 200 yards northeast of Stonecliff, Area 8; soil and humus near cemeteries, Areas 11 and 12, and from water and debris, east edge of Marsh, Area 15. This ubiquitous species has been identified with difficulty. Only the isolate recovered from Area 15 was truly comparable to the species as understood by Coker and Matthews. The other isolates have varied from the type, particularly with regard to the number of oospores, and the size of oogonia. The percentage of antheridia formed in our collections has been so variable, ranging from 15–90%, that it was of little value in the determination of the species of the majority of our isolates. The percentage of antheridia for *S. mixta,* as described by Coker and Matthews, is "40% or more but not on all of the oogonia." None of our isolates has produced cylindrical oogonia in old, discharged sporangia, and it is primarily on this basis that our strains have been separated from *S. ferax,* a closely-allied species.

Saprolegnia delica Coker. Collected from soil and debris near vegetation line at bathing beach, Area 4, and from soil and debris near vegetation line, Pontiac's Lookout, Area 5. Our collections differ from the species as defined by Coker (1) only in the fewer number of small, abnormal oospores formed in the large oogonia. Up to 40 such oospores were reported by Coker; only up to 28 have been observed in the oogonia of our isolates.

Saprolegnia diclina Humphrey. Recovered from soil and humus, Protestant Cemetery, Area 11, and from Military Cemetery, Area 13. The occasional occurrence of oogonia in chains is reported in *S. diclina* by Coker (1), although Humphrey (4) makes no mention of this feature. In the Mackinac Island material of this species, grown under the conditions previously outlined, the appearance of oogonia in a moniliform arrangement is rather infrequent.

The percentage of such organs has been altered from 2–55 by varying the temperature at which the cultures were grown. The highest percentage of moniliform oogonia was obtained in a culture developing at 10 ± .3° C., while the lowest percentage was found in a culture grown at 22 ± .4° C. With respect to the other features which identify *S. diclina,* our isolates agree remarkably well, particularly in the disappearance of the antheridial branches soon after the antheridial cells are abstricted.

Saprolegnia monoica var. *glomerata* (?) Tiesenhausen. This variety was found in water and debris taken from a beach pool at the southeastern end of the bathing beach, Area 3. Our collection resembles quite closely Coker's conception of the variety, but is markedly different from Tiesenhausen's original description (**10**). Three points of distinction between our isolates and Tiesenhausen's may be made. First, the oospore number in the variety *glomerata* is less than in our material. Second, the contortions, clustering, or irregularities of antheridial branches, characteristic of the variety, are not found in the Mackinac Island material. Third, the terminal oogonia of the variety *glomerata* are reported to be usually cylindric in old sporangia, a feature never observed in the isolates which we have collected.

Three non-fruiting members of the genus *Saprolegnia* were collected from beach pool water, west of Grand Hotel, Area 2; from water, west edge of marsh, Area 14, and from soil, margin of Old Fort Garden pool, Area 16.

Achlya americana Humphrey. Recovered from water and debris, bathing beach, Area 4, and from humus in intermittent bog, Area 10.

Achlya flagellata Coker. Collected from soil below Stonecliff, Area 7.

Achlya Rodrigueziana F. T. Wolf. Isolated from soil and debris in Old Fort Garden, Area 16. Our material of this plant resembles the characteristics of the type with the exception of slight variations in the percentages of oospore number, when compared with the percentage of frequency of these structures as reported by Wolf (**11**). This species has also been collected in Illinois (Shanor, personal communication). The Illinois material has characteristics, however, which are not in complete agreement with the type of the species.

Two sterile isolates of *Achlya* were recovered from two collections of water in Area 8, 200 yards northeast of Stonecliff.

Dictyuchus anomalus Nagai. Isolated from soil and humus, intermittent bog, Area 10. This interesting plant has previously been reported from Florida (3), and from North Carolina (Couch, personal communication). It has appeared in several of the writer's collections from the Lower Peninsula of Michigan. The fungus is quite easily recognized by the complete lack of antheridia. Our strain differs from the strains collected by Couch, only in the prolific production of oogonia.

Dictyuchus sp.? Presumably heterothallic strains collected from humus and soil, west edge of marsh, Area 14, and from soil, Scott's Cave, Area 17. No sexual reproductive structures have been induced by any of several cultural conditions to which the plants have been subjected. Sporangia are all of the true-net type. With regard to the sporangial morphology and vegetative characteristics of the isolates, there is an obvious resemblance to *D. monosporus* Leitgeb (*D. sterile* Coker). Several attempts have been made at crossing the Mackinac Island material with other supposedly heterothallic strains of *Dictyuchus*, collected elsewhere in Michigan, but these have met without success. It is assumed, then, that we have only non-complementary strains, and until our isolates can be paired with compatible strains with the resulting production of sex organs, positive identification cannot be made.

Isoachlya, non-fruiting. Isolated from soil near Scott's Cave, Area 17. As in the previous non-fruiting isolates herein listed, vegetative and asexual characters have necessarily been used in the identification of this plant. Considering these features, there is no doubt that our isolate is a member of the genus *Isoachlya*. We have made several attempts to induce sex organs by means of variations of temperature and nutrition, but these have not been successful.

A fungus was recovered which closely resembles *Saprolegnia monoica* Pringsheim, previously reported from Michigan by Pieters

(6, 9). Because of incompleteness of description of *S. monoica,* an extensive taxonomic study of our fungus is being conducted, the results of which are to be reported at a later date.

SUMMARY

The results of a preliminary survey of the Saprolegniaceae in the soil and waters of Mackinac Island, Michigan, are reported.

The fungi recovered are distributed in four genera, and consist of nine previously described species and one variety, and six non-fruiting isolates. A new species of *Achlya*, recovered from the soil of three areas on the island, is described as *Achlya michiganensis.*

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EXPLANATION OF FIGURE 2

Achlya michiganensis

FIG. 2. A. Habit sketch of portion of mycelium, showing the distinguishing features of the fungus when grown on hempseed. B. Primary and secondary sporangia, showing method of spore discharge, and short cymose branching. Secondary sporangium with two lateral discharge tubes. C. Typical gemma. D. Terminal oogonium with immature oospores, and diclinous antheridia, one of which is abnormally short. E. Lateral oogonium with two mature oospores, showing a strongly curved oogonial stalk, and branched, irregular, diclinous antheridia. F. Immature oogonium with both a diclinous and an androgynous antheridium attached. G. Oogonium with three mature oospores, showing a very long, bent oogonial stalk, and a branched, diclinous antheridium. H. Large, abnormal oogonium with ten small, immature oospores, and two diclinous antheridia. All drawings made with aid of Spencer camera lucida. Fig. A, appx. × 620; fig. B, × 410; all others, × 840.

RAMULARIA LEAF SPOTS OF LATHYRUS ODORATUS AND L. LATIFOLIUS

KENNETH F. BAKER, WILLIAM C. SNYDER, AND LILY H. DAVIS

(WITH 2 FIGURES)

In June, 1933, a *Ramularia* leaf spot of sweet pea (*Lathyrus odoratus* L.), and in July, 1938, a similar leaf spot of perennial pea (*L. latifolius* L.), were first collected in California in Santa Barbara County.[1] We have subsequently observed these diseases on many occasions in coastal areas of the state.

The leaf spots resemble closely those of *Lathyrus* spp. attributed in the literature to species of *Scolicotrichum, Ovularia, Ramularia, Cladosporium, Hyalodendron,* and *Erostrotheca.* The question arose, therefore, as to the possible identity of the California fungi with those described elsewhere, and the proper binomial of the pathogen. The conclusions reported here are based on observations of field material, cultures of the fungi, inoculation tests, and examination of the exsiccati cited here, during a period of several years.

SYMPTOMS

On sweet pea the symptoms are the same as those described and illustrated by various workers (**14**, **19**, **37**). On leaves the buff-colored dead areas are circular or irregular and merge gradually into healthy tissue. Sporulation occurs as white tufts visible with a hand lens on both sides of the leaf but most abundantly on the undersurface. The spots vary from small yellow flecks to areas involving most of the leaf, and leaf fall is usually hastened. Lesions may occur on the wings of stems.

On perennial pea the spots occur on leaflets, petioles, stipules, tendrils, stems (FIG. 1, C), peduncles, and pods. They are typically tan to dark brown with definite margins usually darker in color than the center. Commonly the leaf spots are restricted laterally by large veins and tend to be elongated (fig. 1, A, D). The basal parts of plants in seed fields are frequently severely spotted, probably because of the high humidity under the dense plant growth. The conidial tufts on this host appear on both leaf surfaces, but are most abundant on the underside (fig. 1, D, E); they are distinctly pink in color.

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[1] Deposited in the Herbarium, Division of Plant Pathology, University of California, Berkeley, under the accession numbers P 568 (collected by L. M. Massey and M. W. Gardner) and P 695 (collected by W. C. Snyder), respectively.

The disease on sweet pea occurs but is not important in outdoor and clothhouse cut-flower plantings in coastal California. It is less common in seed fields because of the drier conditions of growth.

The disease on perennial pea is much more common and conspicuous in seed fields because of the perennial nature of the plant, the more vigorous growth, and the practice of ditch irrigation. It is, however, unimportant on this minor crop. Lesions on the pods might extend through the pod walls, infecting the seeds, but such seed transmission was not investigated.

There are few diseases of sweet pea or perennial pea in this country that might be confused with *Ramularia* leaf spot. Powdery mildew (*Erysiphe polygoni* DC.) occurs commonly over the plant and forms sparse to dense, superficial, white mycelial growth without fasciculate tufts, and sometimes involves all of the leaf and stem surfaces. The *Ramularia* leaf spots are definitely localized and have dead centers in which small white to pink tufts of conidiophores are evident, without superficial mycelial development.

Anthracnose [*Glomerella cingulata* (Ston.) Spauld. & Schrenk] is said to cause white or pallid leaf spots and to girdle stems, producing dead tips. Acervuli bearing slimy salmon-colored masses of spores occur in these spots. The *Ramularia* disease is not known to kill shoots, and produces dry, pink or white, conidial tufts.

Ascochyta blight [*Ascochyta* spp., *Mycosphaerella pinodes* (Berk. & Blox.) Stone] is reported to produce dark brown to purple lesions on the stems, foliage, and pods, or light brown indefinite spots on the leaves, apparently depending upon the species of *Ascochyta* involved. In all cases, however, light to dark brown pycnidia form in the blighted tissues followed later, in some instances, by perithecia of the *Mycosphaerella* state. The presence of pycnidia which exude their conidia in tendrils, and the lack of aerial development of the fungus on the surface readily distinguish this disease from *Ramularia* leaf spot.

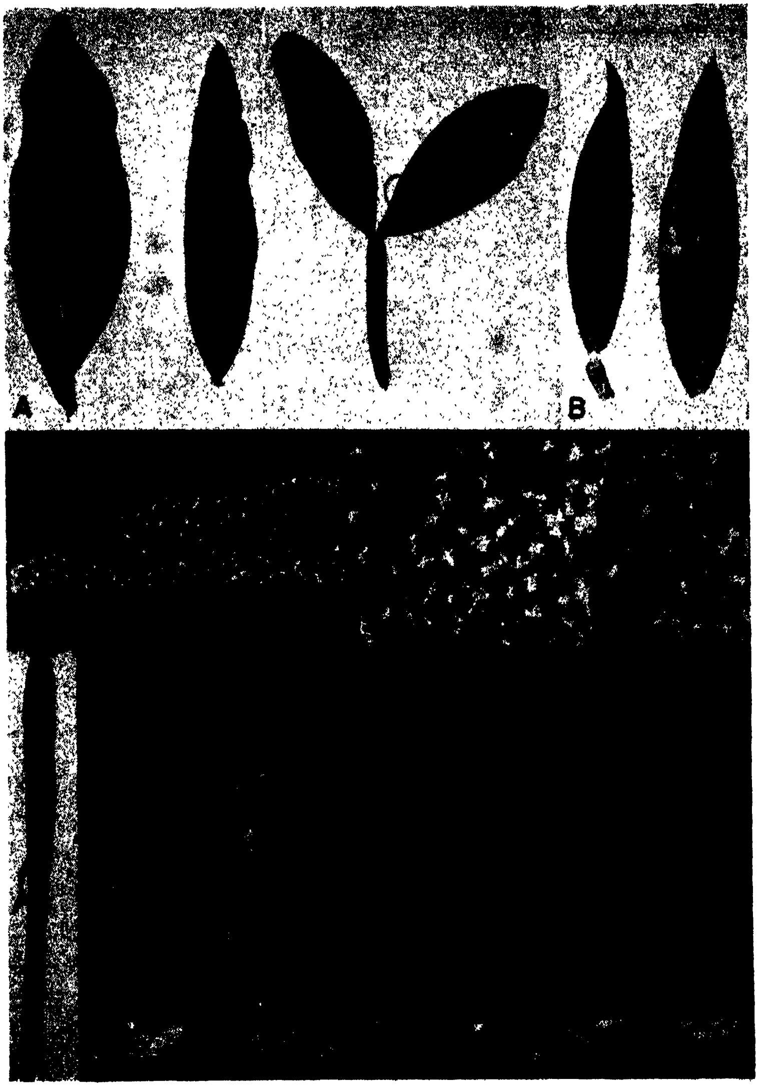

FIG. 1. *Ramularia* leaf spot of perennial pea.

The common name that seems most appropriate for the *Ramularia* diseases is *Ramularia* leaf spot. The disease of sweet pea has been called white blight, white mold, and *Cladosporium* blight.

GEOGRAPHICAL DISTRIBUTION

Pathological studies with the organism were first conducted by Dowson (14) in England in 1922 on sweet pea, but Lind previously had collected it on that host in Denmark in 1913 (Kabát et Bubák, Fungi imperfecti exsiccati No. 791). The disease on this crop was first reported in the United States at Reading, Massachusetts on April 27, 1927 (collection of E. F. Guba), and may have been present by 1922 (3). It was found in the same year in New York, and in Pennsylvania in 1928 (37), in New Jersey from 1932 to 1934 (49), in California in 1933 and in Texas in 1942 (1). It was reported to the Plant Disease Survey by C. E. F. Guterman as *Ramularia* sp. on sweet pea on Long Island, New York in 1933; specimens collected there by him in 1935 were referred to *Cladosporium album.* In Canada it was first seen in 1931 in British Columbia (23), and it was again reported there in 1933, 1944, and 1947; it was found in Ontario in 1945 (19).

An *Ovularia* sp. on perennial pea (*L. latifolius*) was collected in California by G. L. Grisvold and A. D. Cameron in 1943, according to Plant Disease Survey records. *Cladosporium album* was reported on this same host in Washington in 1943 (U. S. Dept. Agr., Mycol. Dis. Surv. herbarium, Forsell and Ramsey, October 29, 1943).

There are many mycological records of what appears to be the same fungus on various *Lathyrus* spp. over much of the temperate and subtropical world. Distribution records given in this paper are summarized below with a reference to the probable earliest collection in each country: Germany in 1869 (17; also Fuckel, Fungi rhenani exsiccati No. 2206); Siberia in 1877 (48): Belgium in 1887 (6); France in 1898–1899 by Fautrey (28); Italy in 1901 (16); Hungary in 1910 (24); Denmark by 1913 (33); Sweden in 1914 (9); Czechoslovakia in 1916 (Petrak, Flora Bohemiae et Moraviae No. 1253); Holland in 1920 (41); England in 1922

(**14**); Caucasus in 1923 (**47**); Alaska in 1923 (Anderson, Alaska Fungi No. 994); Estonia in 1925 (**32**); United States in 1927 (**3**); Lithuania in 1927–1932 (**8**); Poland in 1930 (**18**, p. 203); Canada (British Columbia) in 1931 (**23**); Ireland in 1933 (**39**); Latvia in 1934 (K. Starcs Herbarium No. 2337); and Scotland in 1942 (**12**). The evidence suggests that the fungus may be native to Europe and Asia.

HOST RANGE

The host plants are given below with a reference to the first apparent record on each: *Lathyrus tuberosus* L. (**17**); *L. pratensis* L. (**48**); *L. sylvestris* L. (Fautrey, *in* **28**); *L. hirsutus* L. (**16**); *L. odoratus* L. (Kabát et Bubák, Fungi imperfecti exsiccati No. 791); *L. maritimus* (L.) Bigel. (**9**); *L. roseus* Stev. (**47**); *L. palustris* L. (Anderson, Alaska Fungi No. 994); and *L. latifolius* L. (Sydow, Mycotheca Germanica No. 2435).

Plants outside of the genus *Lathyrus* have been reported as hosts of this fungus. Among these are: *Lotus* (Tetragonolobus) *siliquosus* L. (**34**); *Vicia* sp. ("Chickling vetch") (**15**); *Vicia* sp. (**40**); *Pisum* sp., *Phaseolus* sp., and *Vigna* sp. (**21, 22**).

Good (**19**) was unable to establish infection with the sweet pea fungus on *Lathyrus latifolius, Vicia faba* L., *V. villosa* Roth., *Melilotus alba* Desr., *Medicago sativa* L., *Pisum sativum* L., *Phaseolus vulgaris* L., *Trifolium pratense* L., or *Glycine max* Merr. Dowson (**14**) was unable to infect *Pisum sativum* and *L. aphaca* L. These unsuccessful inoculations may be explained on the basis of the existence of physiologic races (**19**).

NOMENCLATURE OF THE FUNGUS

There are a number of possibilities to be considered in the selection of the most appropriate binomial for this fungus. These are discussed here chronologically.

1. *Ovularia fallax* (Bon.) Sacc. This fungus, described by Bonorden (**7**) as *Crocysporium fallax* Bon. on *Vicia* sp., was placed by Saccardo (**43**, vol. 13: 1307) in Ovularia. It is now known (**35, 44**) from examination of specimens that this was the conidial stage of a powdery mildew, and is, therefore, invalid.

2. *Ovularia deusta* (Fuckel) Sacc. This fungus was described by Fuckel (**17**) as *Scolicotrichum deustum* Fuckel, causing deep brown to black spots on living leaves of *Lathyrus* (Orobus) *tuberosus* in the fall at Eberbach, Germany. Saccardo (**43**, vol. **4**: 140–141) placed this under *Ovularia deusta* and repeated Fuckel's description practically verbatim. The specific name *deusta* (from the Latin *deustus,* burnt) may have been used because of the dark appearance of the spots or perhaps because of the tiny, pink, conidial clusters conspicuous in Fuckel's specimen. The spores were 4 × 12 μ, lanceolate, continuous, and hyaline; conidiophores were fasciculate, pink, simple, slender, and bore terminal conidia.

The binomial *O. deusta* has been subsequently used by many workers down to the present day. Among these are: Bommer and Rousseau (**6**) in Belgium in 1887; Kirchner (**30**, pp. 143 and 439) in Germany in 1890; Knet (Fungi Schemnitzienses, collected in Germany by Sitno, August 20, 1897); Lindau (**34**) in Germany; Lind (**33**, p. 499) in Denmark; Briosi in Italy in 1912 (**2**); Marchal (**36**) in Belgium between 1913–1919; Petrak (Flora Bohemiae et Moraviae No. 1253) in Czechoslovakia in 1916; Jaap (**27**; also Fungi selecti exsiccati No. 844) in Germany in 1916; Siemaszko (**47**) in the Caucasus in 1923; Sydow (Mycotheca Germanica No. 2224 [FIG. 2, I] and No. 2435) in Germany in 1923 and 1924; Brundza (**8**) in Lithuania between 1927 and 1932; Hruby (**25**) in Czechoslovakia in 1928; Garbowski and Juraszkówna (**18**, p. 203) in Poland in 1930; Eriksson (**15**, p. 444); O'Connor (**39**) in Ireland in 1933; Muskett *et al.* (**38**) in Ireland in 1934; and Chesters (**10**) in England in 1937.

Examination of Fuckel's Fungi rhenani exsiccati No. 2206 in the Farlow Herbarium [2] showed this organism (FIG. 2, H) to be the same as the one that occurs in California. The legend on this specimen is as follows: "2206. *Scolicotrichum deustum.** Ad Orobi tuberosi folia marcescentia frequens. Autumno. *Von allen, hier neu aufgestellten, aber nicht beschriebenen, Arten und Gattungen befinden sich die ausführlichen Beschriebung in meinen im Drucke befindlichen 'Symboliae mycologicae.' "

3. *Ramularia Galegae* Sacc. f. *Lathyri* Ferraris. The species

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[2] Examined through the courtesy of Dr. W. L. White.

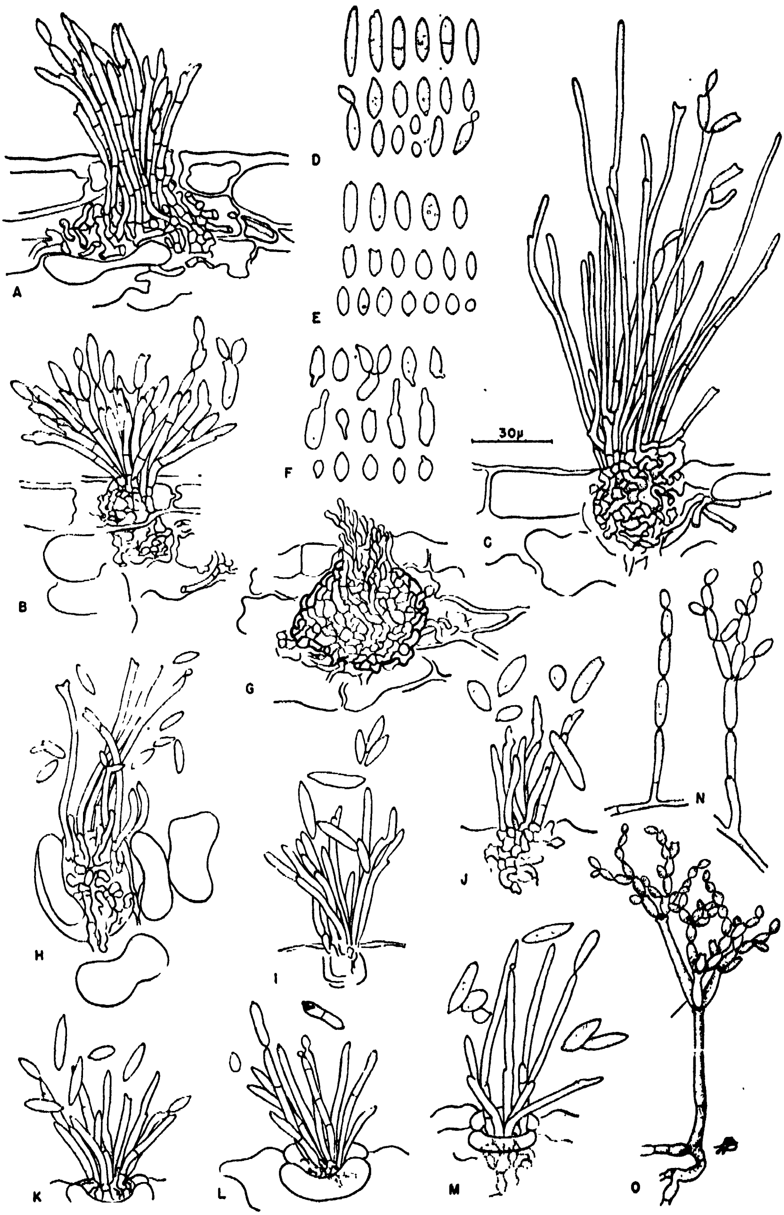

FIG. 2. *Ramularia* leaf spot of perennial pea.

was described by Saccardo (42) on *Galega officinalis* L. in Italy. The form was reported on *Lathyrus hirsutus* L. in Italy in 1901 (16) producing spots which lacked definite margins. The conidiophores were fasciculate and hyaline; the conidia were continuous or rarely 2-celled, hyaline, 16–26 × 3–3.5 μ.

The fungus was said to have been collected by Fautrey on *L. sylvestris* in France in 1898 and exsiccati were distributed as Herbier Cryptogamique de Côte d'Or No. 2630. Ferraris later changed his original binomial to *R. Lathyri* Ferr. (28). The situation was reviewed by Keissler (28) and the conclusion reached that *R. Lathyri* Ferr., *R. Lathyri* (Fautr.) Ferr., *R. Galegae* f. *Lathyri* Fautr., and *R. Galegae* f. *Lathyri* Fautr. apud Ferr. are all synonymous.

Bubák (9) also considered *R. Galegae* Sacc. var. *Lathyri* Ferr. and *R. Lathyri* Hollós, both on *Lathyrus hirsutus,* as synonymous and changed the former to *R. Lathyri* (Ferr.) Bubák.

Lind collected *R. Galegae* Sacc. f. *Lathyri* Ferr. on *Lathyrus odoratus* in Lyngby, Denmark on September 28, 1913 (Kabát et Bubák: Fungi imperfecti exsiccati No. 791). This appears to be the first record of the fungus on cultivated sweet pea. Starcs found the fungus in 1934 in Latvia on *L. odoratus* and called it *R. lathyri* Ferr. (K. Starcs Herbarium No. 2337).

All of these names are later than *Ovularia deusta.* Examination of exsiccati of Kabát and Bubák (FIG. 2, J) and Starcs shows that they do not differ significantly from the California fungus.

4. *Ramularia Lathyri* Hollós. This fungus was described on *L. hirsutus* in Hungary in 1910 (24). The conidiophores were fasciculate and hyaline, 40–50 × 3 μ; the conidia cylindrical or sometimes obtuse, catenulate, continuous or 2-celled, hyaline, 16–20 × 4–5 μ. The binomial *R. Lathyri* Hollós was used by Siemaszko (47) for a fungus on *Lathyrus roseus* Stev. in the Caucasus in 1923.

5. *Ramularia roseola* Bubák et Vleugel. This species was applied to a *Ramularia* on *L. maritimus* (L.) Bigel. in Sweden in 1914 (9). The leaf spots possessed definite margins and the tufts of conidiophores were pink. Conidiophores were fasciculate and 28–56 × 2.5–3 μ. Conidia were catenulate, hyaline; microconidia

were continuous, 9–13 × 2.5–3.5 μ; macroconidia cylindrical, 19–28 × 2.5–4 μ, continuous to uniseptate. This binomial was applied by Cash to a collection made by Anderson (Alaska Fungi No. 994; FIG. 2, L) on *L. palustris* in 1923. Examination by us of Anderson's specimen showed that it differed in no significant particular from the other fungi under consideration.

6. *Cladosporium album* Dowson. This binomial was applied in 1924 (**14**) to a fungus that caused leaf spots of *Lathyrus odoratus* in England. The types of lesions produced and the characteristics of the fungus on the host and in culture agree with those of the California pathogen. Apparently in classifying this fungus greater emphasis was placed on its appearance in culture than on the host, and the taxonomic importance of color was minimized. Leaf specimens were stated to have been independently examined by Miss E. M. Wakefield and Dr. J. Ramsbottom and considered to be an unusual form of *Ramularia*. Cultures were examined by Prof. F. T. Brooks and said to resemble *Cladosporium herbarum* except in color. Examination by us of Dowson's exsiccati (Coll., Surrey, England by Dowson, Oct. 23, 1924) showed that *C. album* (FIG. 2, K) did not differ from Fuckel's specimen or the California material. Good (**19**) minimized the differences between *C. album* and *C. fulvum* Cke. in culture prior to sporulation. Because *fulvum* mutated to a hyaline form, he also felt that color was of little fundamental importance. There are marked differences between colonies of the *Lathyrus* fungus and *C. fulvum* in growth rate and habit, maximum size attained, and color (FIG. 1, F–H), as well as in morphology (FIG. 2, N. O).

There are close similarities of spore formation in culture by the sweet-pea fungus [Fig. 50 of Dowson (**14**) and Fig. 7 of Good (**19**)], established *Ramularia* spp. [Figs. 9–12 of Laibach (**31**), Fig. 20 of Hughes (**26**), and Fig. 9 of Gregory (**20**)], and established *Cladosporium* spp. [FIG. 2, O of the present paper], particularly when they are examined under low magnification. However, careful studies of *R. deusta* under high magnification reveal a tendency toward fasciculation in culture, and the spores tend to form more readily into long chains than in clusters as in *Cladosporium*. The character of the scars and the shape of the conidiophore tips as well as the connections between conidia are quite distinctive (FIG. 2, N, O). Morphology of the fungus in culture obviously cannot be used alone for identification of these genera. Because of these considerations, the designation of the *Lathyrus* organism as a *Cladosporium* obscures its true affinities both as an entity and as a pathogen.

Seymour (46) questioned "the accuracy of the determination" of the reported *C. album* in North America. Following a discussion with the senior author, Savile (11) stated that this fungus should be regarded as a *Ramularia* rather than a *Cladosporium.*

Classification of many Fungi Imperfecti is based in large measure on their sporulation on the host. Some of the Melanconiales, for example, produce no acervuli in culture and cannot be correctly identified unless observed in their parasitic state. Of pathologists and mycologists who examined the white-blight fungus on the host for us, several placed it in *Ramularia* or *Ovularia* but no one in *Cladosporium.* The older name, *Ovularia deusta,* applied to the same fungus more nearly expresses its relationships.

Dowson (14) emphasized that the indefinite margins of the leaf-spot on sweet pea were atypical for *Ramularia,* but were known for *C. fulvum* on tomato. Similar diffuse leaf spots are caused by *R. Vallisumbrosae* on narcissus (20) and *R. cyclaminicola* on cyclamen (4). The form of *Ramularia deusta* on perennial pea consistently produces definite dark-margined leaf spots, as apparently is the case on wild *Lathyrus* spp. Possibly a diffusible toxin is formed during invasion of sweet pea but not of other *Lathyrus* spp. Spots on leaves of sweet pea often lose their diffuse character when pressed and dried, showing necrotic areas with almost as sharp a margin as those on perennial pea.

The binomial *C. album* has been commonly used in Europe and North America [*e.g.,* 1, 3, 5, 12, 19, 21, 23, 32, 37, 40, 41, 45; Guterman (Cornell University Department of Plant Pathology herbarium No. 24750); Cash (Forsell and Ramsey in Washington, October 29, 1943, U. S. Dept. Agr. Mycol. Dis. Surv. herbarium)].

7. *Erostrotheca multiformis* Martin and Charles. This name was applied (37) in 1928 to a fungus that was thought to be the perfect stage of *C. album.* Good (19) has expressed doubt about

this relationship because of differences in morphology and colony size between this fungus and *C. album* in culture.

There are several lines of evidence that lead to the conclusion that *E. multiformis* is not connected with *C. album.* The results of Dowson, Good, and the writers are in agreement that the causal organism is extremely slow-growing, producing colonies about 10 mm. in maximum diameter from single conidia (FIG. 1, G, H). A single-spore culture is not macroscopically evident for at least a week. *Erostrotheca* was illustrated and described (**37**) as rapidly producing luxuriant zonate colonies about 40 mm. across and forming the perfect stage in 6 days.

Evidence is lacking in the original paper (**37**) that any of their 7 "stages" of the fungus in culture (*Cladosporium, Hormodendron, Ovularia, Haplaria, Pseudofumago, Pseudosaccharomyces, Erostrotheca*) was tested for pathogenicity. Because of the uncertain status of these various forms, it is not clear that the true pathogen was even represented among the imperfect forms observed in culture or that they had any connection with the sexual stage. Examination of leaves from their material[3] collected on Long Island, New York, on May 24, 1927, and labeled "original culture made from *Cladosporium* stage on this material" has established the identity of their fungus, *C. album* (FIG. 2, M), with *Ramularia deusta* in California. Martin and Charles (**37**) stated that specimens from Pennsylvania, Massachusetts, and from Dowson in England proved to be the same fungus (*C. album*) as their New York material. An ascigerous fungus was obtained by them in one isolate out of 15, and this culture was used in all studies. It seems probable that the *Erostrotheca* overgrew and suppressed the slow-growing *C. album.* Slow development of small colonies in culture is usual for *Ramularia* and *Ovularia* (**20, 26, 29**) but not for *Cladosporium.* We have cultured various *Ramularia* and *Ovularia* species (*e.g., Ramularia cyclaminicola* Trel. from cyclamen, *R. decipiens* Ell. & Ev. from Rumex, *R. pastinacae* Bub. from parsnip, *R. lactea* (Desm.) Sacc. from Viola, *R. cynarae* Sacc. from artichoke, *R. primulae* Thüm. from Primula, *Ovularia* sp. from vetch) and all produced slow-growing colonies similar in appearance to *R. deusta.*

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[3] Specimen obtained through the courtesy of Dr. W. D. McClellan and Dr. P. Brierley.

Erostrotheca is a member of the Hypocreales. All *Ramularia, Ovularia,* or *Cladosporium* spp. known to have a perfect stage are in Mycosphaerella of the Sphaeriales. We have been unable to find any record of observation or collection of the genus *Erostrotheca* since its erection.

In order to clarify this situation we have for some years searched for the perfect stage of the two strains of *Ramularia deusta* in California. Cultural studies and examination of field material at various seasons have failed to provide more than a suggestion that one exists. During the winter months the *latifolius* form frequently produced in substomatal cavities of leaves sclerotia-like structures which bore conidiophores on the upper surface (FIG. 2, C, G). These structures resemble those described (20) for *Ramularia Vallisumbrosae* Cav., and similarly may represent immature perithecia. On the other hand, it is equally possible that these are sclerotia functioning solely for aestivation as reported for *R. onobrychidis* (26). Structures that were perhaps spermagonia have been reported (20, 26) without clear genetical connection with these two fungi. Similar structures resembling spermagonia were present on old perennial pea leaves in winter in California, but it was not demonstrated that they were a stage of *R. deusta.* Their presence suggests the possible existence of a perfect stage of the *Mycosphaerella* type. Good (19) reported structures for the sweet pea strain in culture that also suggested spermatia and perithecial initials. Laibach (31) and Killian (29) reported the formation of sclerotia in numerous *Ramularia* spp. Production of "sporodochia" by the sweet-pea fungus (FIG. 2, A) is consistent with their demonstrated formation (20, 26, 29) by various *Ramularia* spp. during the summer months. These similarities of the *Lathyrus* fungus to known Ramularias support the close relationship to that genus presented in this paper.

In consideration of these facts, the name *Erostrotheca multiformis* is set aside pending evidence that it is the perfect state of *C. album.*

8. *Hyalodendron album* (Dowson) Diddens. *Cladosporium al-*

bum was transferred to this genus by Diddens (13) at the time it was erected for a saprophytic wood-pulp fungus. *C. album* was studied in culture and considered to be better included in *Hyalodendron* than left as a hyaline *Cladosporium*. There is no evidence that the fungus was studied on its host. This transfer is rejected because it obscures the relationship to other leaf-spotting *Ramularia* and *Ovularia* spp. and would impede practical identification of the fungus, and because *Ovularia deusta* has nomenclatural priority.

9. *Ramularia deusta*. The binomial, *Scolicotrichum deustum* Fuckel, has priority over other names applied to this fungus. Because Fuckel described and correctly placed species in the moniliaceous genus *Ramularia* and the dematiaceous genus *Scolicotrichum* in his Symbolae Mycologicae, it is inexplicable why the *Lathyrus* fungus was placed in *Scolicotrichum*. Certainly the exsiccati and his description leave no doubt that the fungus he named *S. deustum* was the *Ramularia* treated in this paper. This error was recognized by Saccardo and the fungus transferred to *Ovularia* in 1886.

The basis for distinguishing *Ovularia* from *Ramularia* is imperfectly defined and there is question whether they should be separated at all (26). *Ramularia* was erected by Unger in 1833 and *Ovularia* by Saccardo in 1880, and should a future monographer combine these genera, the older name would be retained.

In the morphological characters proposed for separating these genera, our fungus more nearly approaches *Ramularia* than *Ovularia*. Ramularias are considered to form spores in long chains as does our fungus, whereas Ovularias bear them singly or in short chains. Numerous Ramularias have been shown (20, 26, 29, 31) to develop sclerotia, as does our fungus (FIG. 2, C, G), while these structures are absent or inconspicuous in *Ovularia*. The pathogen is nearer to *Ramularia* than to *Ovularia* because of its cylindrical spores. The degree of roundness of the spores has been found to vary with fresh *vs.* dried material, on host *vs.* in culture, and on the host under dry *vs.* moist conditions (FIG. 2, D, E) as reported for other Ramularias (20, 26). The germination of conidia is shown in figure 2, F.

Under moist conditions, some Ramularias tend to form in the conidial apparatus elongate structures [4] that have been regarded (20) as scolecospores, but can as logically be interpreted as phialides of the conidiophores. They are much less common in *Ovularia* than *Ramularia*. The diverse collections of *R. deusta* examined showed all gradations of development of these structures (FIG. 2, A–C, H–M).

Because of these considerations, the fungus is transferred from *Scolicotrichum* to *Ramularia*. An emended description and the synonomy of this pathogen are as follows:

Ramularia deusta (Fckl.) n. comb.

Synonomy:

Scolicotrichum deustum Fuckel, 1869.
Ovularia deusta (Fuckel) Sacc., 1886.
Ovularia deusta Sacc., 1887 (6).
Ramularia Galegae f. *Lathyri* Fautrey, 1898-99 (28).
Ramularia Galegae Sacc. f. *Lathyri* Ferraris, 1906.
Scolecotrichum deustum Fuckel, 1907 (34).
Ramularia Lathyri Ferraris, 1910 (28).
Ramularia Lathyri Hollós, 1910.
Ramularia Lathyri (Ferr.) Bubák, 1916.
Ramularia roscola Bubák et Vleugel, 1916 (9).
Ramularia Galegae f. *Làthyri* Fautr. apud Ferraris, 1923 (28).
Ramularia Lathyri (Fautr.) Ferraris, 1923 (28).
Cladosporium album Dowson, 1924.
Hyalodendron album (Dowson) Diddens, 1934.

Nomen ambigum: *Erostrotheca multiformis* Martin and Charles, 1928.

Conidiophores densely fasciculate, typically unbranched, slender, hyaline, continuous or with several septa, highly variable in length, 20–60 × 3–4 μ [usually 50 × 3–4 μ; under moist conditions develop elongate structures (conidiophores or "scolecospores") which extend the total length up to 200 μ], bear terminal conidia, and become geniculate with age. Conidia cylindrical, with tapering ends, continuous or 2-celled, hyaline, minutely echinulate, 2–28 × 1–5.5 μ, borne in acropetalous fashion, forming short chains which may be branched.

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[4] Since this paper was submitted for publication, my attention has been called to an article by Dr. W. C. Moore in which a similar situation in *Ramularia primulae* is discussed (Trans. Brit. Mycol. Soc. 25: 208–209. 1941).

PHYSIOLOGIC FORMS

Slight differences in colony appearance between the sweet pea and perennial pea isolates and the differences in color of sporulation and symptom expression on these hosts suggested the possibility that biologically specialized races of the fungus were involved in these two diseases. To test this possibility, sweet peas and perennial peas grown in pots were cross inoculated by atomizing them with spore suspensions of the organisms produced in culture. Although only one such test was made, the results indicated that the two isolates were biologically distinct. Typical leaf spots and stem lesions were obtained on *L. latifolius* only with the isolate from that host (FIG. 1, B). Likewise, infection of *L. odoratus* occurred only when the sweet pea isolate was used. The controls were not infected. It is concluded that the *Ramularia* leaf spots of sweet pea and perennial pea are caused by distinct races of *Ramularia deusta.*

Inoculation data available, including that reported by Good (**19**), plus the known host specificity of Ramularias and Ovularias, indicate the existence of a number of physiologic forms of *R. deusta.* It would seem best to designate these as formae of the morphologic species, *R. deusta,* as they become known through inoculation tests. Accordingly, we propose two forma names to distinguish between the strains which attack sweet pea and perennial pea: **Ramularia deusta** f. **odorati** n. f. and **Ramularia deusta** f. **latifolii** n. f., pathogenic and causing leaf spots upon *Lathyrus odoratus* and *Lathyrus latifolius,* respectively.

DISCUSSION

In an admittedly artificial system of classification such as that of the Fungi Imperfecti, the convenience and accuracy of identification of specimens should be a major consideration. It is important to pathologists that an imperfect fungus be so placed that other workers will readily recognize the relationship of their organism to it. The nomenclatural situation discussed in this paper shows the confusion that results from parallel usage of different names for the same organism. A similar situation is discussed elsewhere (**4**) for *Ramularia cyclaminicola* Trel. The established basis for classification of fungi of this type is their morphology on the host with only secondary consideration to cultural characters. The fungus considered here is a *Ramularia* in its parasitic state and is therefore transferred to that genus.

The spore measurements of the majority of descriptions and specimens that we have examined fall within the fairly consistent range of 2–20 × 1–5.5 μ. However, descriptions of two of the binomials here considered (*Ramularia Galegae* Sacc. f. *Lathyri* Ferraris and *Ramularia roseola* Bubák et Vleugel) have somewhat larger spores. Despite this difference, the former has been considered (**9**, **28**) as synonymous with the smaller spored *R. Lathyri* Hollós. Because this range includes measurements of spores produced on culture media, numerous *Lathyrus* species, and under different moisture conditions (**20**, **26**), the variation in conidial size is not regarded as significant. The range of variation (2–28 × 1–5.5 μ) of all collections of *R. deusta* discussed here is small compared with that demonstrated (**20**, **26**) for *R. Vallisumbrosae* (3–44 [scolecospores up to 105 μ] × 2–4 μ) and for *R. onobrychidis* (7–42 × 2.7–6.3 μ).

Other *Lathyrus* pathogens discussed here are considered to be the same as Fuckel's *deusta,* which is here transferred to *Ramularia.* This specific name has the advantage of almost continuous usage since 1869. The use of the forma names, *odorati* and *latifolii,* to denote host specificity permits retention of the morphologic species *R. deusta,* while distinguishing between its physiologic forms.

SUMMARY

Evidence is presented that certain *Lathyrus*-infecting fungi of the genera *Scolicotrichum, Ovularia, Ramularia, Cladosporium,* and *Hyalodendron,* including the pathogens causing white blight or white mold of sweet pea and a previously undescribed leaf spot of perennial pea, should be designated as *Ramularia deusta* (Fckl.). Other specified binomials, including the commonly used *Cladosporium album* Dowson, become synonyms.

It is concluded that the relationship of the Ascomycete, *Erostrotheca multiformis* Martin and Charles, to the sweet pea Hyphomycete has not been demonstrated, and it is improbable that they

are states of the same fungus. This name is therefore set aside, pending evidence of such genetical connection.

The situation discussed here illustrates how parallel use of different names for the same fungus, arising from incomplete information of the pathogen's development on the host and in culture, results in confused nomenclature and obscured relationships.

Ramularia deusta is widespread over the temperate and subtropical world on 9 species of *Lathyrus,* including the cultivated *L. odoratus* and *L. latifolius.* Physiologic specialization is probably of common occurrence in *R. deusta.* Two cases are recognized here by the proposal of two new forma names, i.e., *R. deusta* f. *odorati* n. f. for the pathogen of *Lathyrus odoratus,* and *R. deusta* f. *latifolii* n. f. for the pathogen of *L. latifolius.*

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DESCRIPTION OF FIGURES

FIG. 1. *Ramularia* leaf spot of perennial pea. A, C, Natural infections of leaves and a stem, respectively. B, Infections resulting from atomizing with spores from a pure culture. D, E, Magnified conidiophore tufts on leaves. F, Twenty-day-old cultures of *Cladosporium fulvum* on potato dextrose agar,

grown from single conidia in light at laboratory temperature. G, Twenty-day-old cultures of *Ramularia deusta* f. *latifolii* comparable to F. H, Sixty-day-old cultures of *R. deusta* f. *latifolii.*

FIG. 2. Camera-lucida drawings. A–G, N, *Ramularia deusta* (Fckl.) n. comb. collections on perennial pea. A, B, Conidiophores developed in summer, A showing a "sporodochium." C, Elongated conidiophores developed in winter from a sclerotium. D, Conidia developed under dry conditions. E, Conidia developed under moist conditions. F, Germinating conidia on water agar. G, Sclerotium developed in leaves in winter. H, *Scolicotrichum deustum* Fckl. on *Lathyrus tuberosus* from Fuckel's Fungi rhenani exsiccati No. 2206. I, *Ovularia deusta* (Fckl.) Sacc. on *L. sylvestris* from Sydow's Mycotheca germanica No. 2224. J, *Ramularia Galegae* Sacc. f. *Lathyri* Ferr. on *L. odoratus* from Kabát and Bubák's Fungi imperfecti exsiccati No. 791. K, *Cladosporium album* Dowson on *L. odoratus* from Dowson's collection, Surrey, England, Oct. 23, 1924. L, *R. roseola* Bub. & Vleug. on *L. palustris* from Anderson's Alaska Fungi No. 994. M, *C. album* on *L. odoratus* from Charles and Martin's collection, North Baldwin, Long Island, N. Y., May 24, 1927, labeled *Erostrotheca multiformis* Martin & Charles. N, *R. deusta* from culture grown on water agar plus cold-sterilized ground aster stems, in light at laboratory temperature. O, *C. fulvum* from cultures comparable to N.

DESCRIPTIONS OF TWO LUMINOUS TROPICAL AGARICS (DICTYOPANUS AND MYCENA)

E. J. H. Corner *

(with 3 figures)

The agarics here described were found by Dr. Y. Haneda and myself in the Singapore Botanical Gardens during the years 1943–1945 when he had charge of the Raffles' Museum. Dr. Haneda had been interested in bioluminescence for some years and he brought to my notice his publications, in Japanese, on luminous fungi from Palao Island in the Pacific. We discovered several of these fungi in Singapore and this paper is the outcome of our preliminary investigations. It was written towards the end of 1944, and in 1949 one copy came through Dr. Haneda in Japan to Dr. A. H. Smith, who kindly returned it to me for revision. One new species, *Dictyopanus luminescens,* belongs to the luminescent alliance of *Panus stypticus* and the other is a new variety, *Mycena rorida* var. *lamprospora,* which presents the hitherto unknown case, according to a verbal communication from Dr. E. C. Wassink, of luminous discharged spores without any part of the fruit-body itself being luminous. Dr. Wassink is arranging for the translation of the copies of Dr. Haneda's papers, which contain much of great interest to students of bioluminescence in general.

I have much pleasure in expressing my gratitude to Dr. Haneda, Dr. Smith, and Dr. Wassink for their cooperation, but it is with great regret that I have to record at the same time the loss of all the manuscript that Dr. Haneda himself prepared during his tenure of office in Singapore.

Dictyopanus luminescens sp. nov. (Figs. 1 and 2)

Fructus toto noctilucens, minutus, lateraliter stipitatus, gregarius, albus demum sordide flavescens vel alutaceus.

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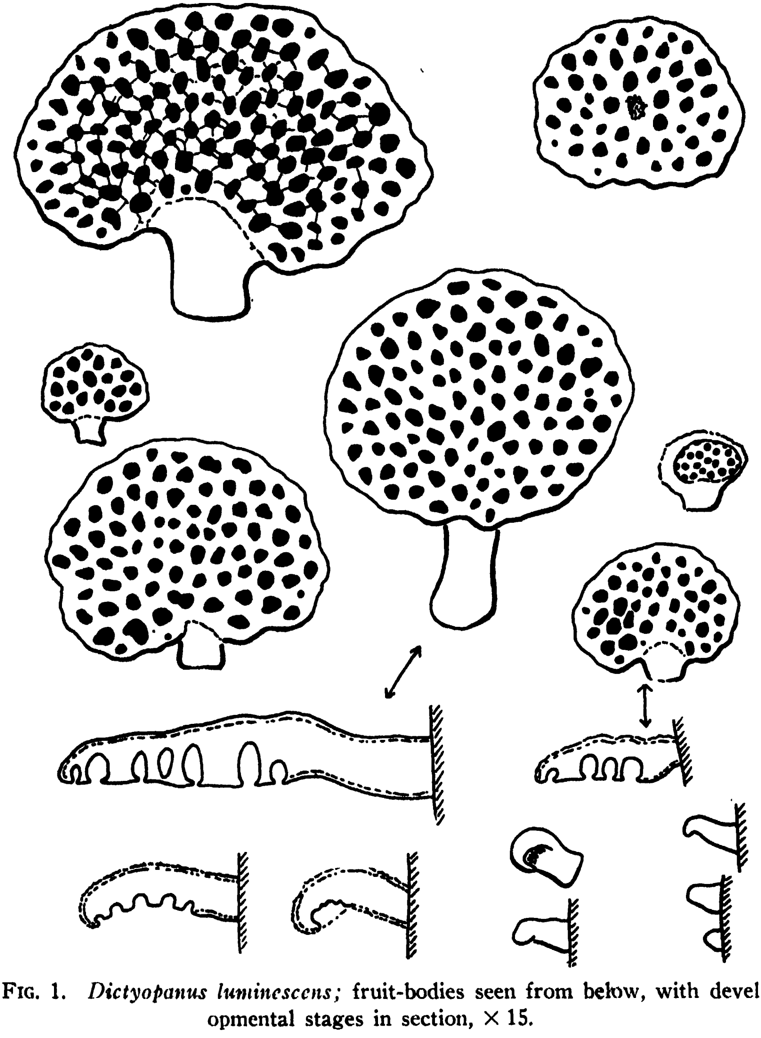

FIG. 1. *Dictyopanus luminescens;* fruit-bodies seen from below, with developmental stages in section, × 15.

Pileus 2–4 mm. latus, convexus demum explanatus, reniformis, toto minute cretaceo-pruinosus, opacus, siccus, demum cute minute fracto: margine integro, incurvato demum explanato.

Stipes 0.1–1.3 × 0.1–0.5 mm., brevis, lateralis (raro subcentralis, ut pileo cretaceo-pruinosus.

Tubuli ad 350 μ longi, 200–250 μ lati, paullum radiati, distincti, nec decurrentes nec lamellosi: pori 70–150 μ lati, constricti, cretaceo-albi: dissepimenta crassa, 50–80 μ lata, tumida, demum minute fracta.

Caro 100–350 μ crassa, alba, sicca, paullum coriacea.

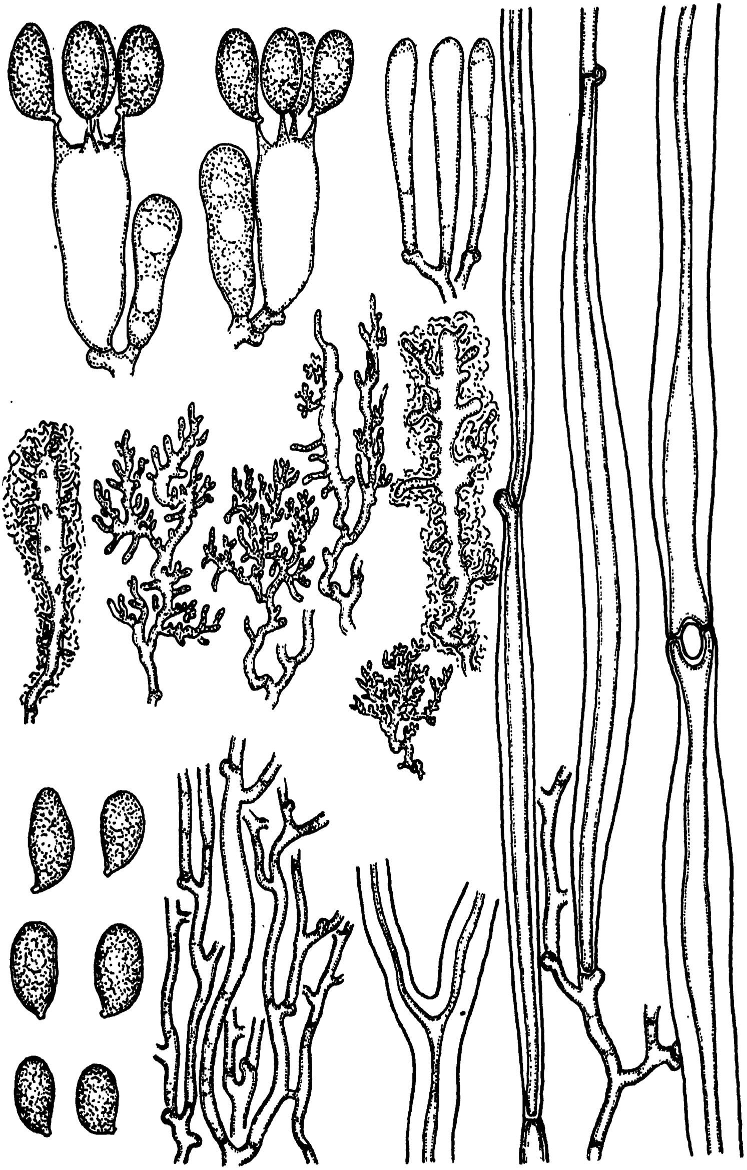

FIG. 2. *Dictyopanus luminescens;* spores and basidia, × 1000; hyphae from the pileus and the base of the stem (thick-walled), × 500; cheilocystidia (clavate) and spiculiferous hyphal ends from the surface of the pileus, × 500.

Sporae 9–13 × 5–6 μ, vulgo 10–11.5 × 5.5 μ, vel minores 7.5–9 × 4–4.5 μ, albae, ellipsoideae, elongatae, leves, aguttatae, parietibus amyloideis.

Basidia 20–28 × 9–10 μ, vel minora 18–25 × 7.5–8.5 μ (dein, sporis minoribus), strato continuo instructa: sterigmata 4, 5–6 μ longa.

Cheilocystidia 30–55 × 7–10 μ, subclavata, tenuiter tunicata, ad margines tubulorum: pleurocystidia nulla.

Hyphae simplices, 1.5–4 μ latae, furculatae, ad superficies fructi angustissimae, dense et intricater coralloideo-ramosae vel spiculiferae, plerumque granulis hyalinis dense incrustatae: in stipite aliae cellulis inflatis 100–250 × 6–20 μ et crasse tunicatae, parietibus ad 7 μ crassis.

Hab. ad folia palmarum emortua (*Rhapis, Pinanga, Arenga*) in silvis: Malaya (? Asia tropicali et Australasia): *typus,* leg. Corner, Singapore Botanical Gardens, 1944.

This is a common Malayan fungus, to be found every rainy season on the dead leaves of palms and, in the Singapore Botanical Gardens, particularly on the dead persistent sheaths of the erect stems of *Rhapis flabelliformis.* Dr. Haneda showed me the bright green phosphorescence of the whole fruit-body and told me that it was probably the fungus recorded as *Polyporus rhipidium* Berk. from the Bonin Islands by Kobayashi (**1937**).

The genus *Dictyopanus* has recently been revised by Singer (**1945**). *D. luminescens* is near to *D. Copelandii,* on dead grasses in the Philippines, and to *D. pusillus* of the New World, but it is characterized by the short stem, the never lamellate pores, the large spores and, perhaps, the luminescence. Singer regards *Polyporus rhipidium* as a variety of *D. pusillus* (= *Polyporus pusillus* Lev.). Now, in addition to Kobayashi's record of luminescence in *P. rhipidium,* Wassink (**1948**) has recorded the mycelium of a strain, so named, from Australia as luminous in culture in Holland. Following Berkeley's original suggestion that *P. rhipidium* resembled *Panus stypticus,* Wassink realized the affinity of the two from their luminescence, but it is possible that both his culture from Australia and Kobayashi's fungus from the Bonin Islands were the large-spored *D. luminescens,* the spore-contents of which shrink on drying to a small lump about the size of the spores of *D. pusillus* (3.5–5 × 1.5–2.5 μ). The spores of *D. Copelandii* are intermediate, 6.8–7.8 × 3.5–4.2 μ, but the pores of this species are characteristically lamellate at the stem-apex.

Dictyopanus differs from *Panellus,* in which genus *P. stypticus* is now placed, merely in the poroid hymenium, but this feature is not constant in the American *D. pusillus,* which may be more or less poroid-lamellate and so approaching the state of *P. stypticus* with fine reticulations at the base of the gills. A revision of the tropical species of the two genera will probably show the necessity of merging them into one, for which luminescence may be a feature.

Mycena rorida (Fr.) Quél. var. **lamprospora** var. nov. (FIG. 3)

A typo differt lamellis principalibus paucioribus 9–14: sporis minoribus 5.7–10 × 3–4.7 μ, noctilucentibus: basidiis semper 4-sporigeris: cheilocystidiis saepe irregulariter lobatis: pileocystidiis grandis, 25–65 × 15–40 μ, clavato-pyriformibus vel subglobosis.

Hab. ad folia et ramulos emortuos in silvis tropicalibus: Asia (Malaya, Ceylon, Rabaul), America (Brazil).

Typus: leg. Corner, Singapore Botanical Gardens, 14 Nov. 1944.

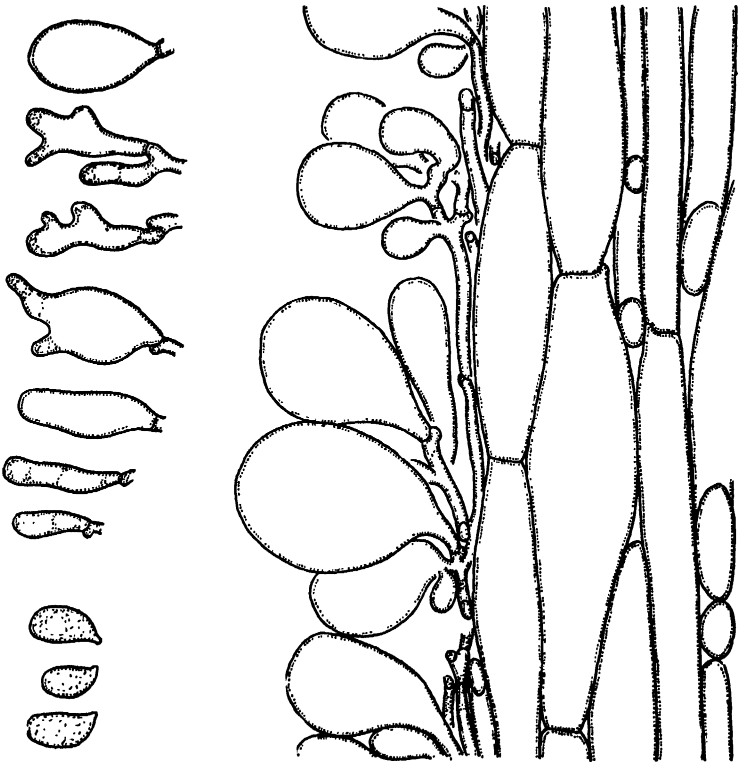

FIG. 3. *Mycena rorida* var. *lamprospora;* spores, × 1000; cheilocystidia and pileocystidia with the adjacent tissue of the pileus, × 500.

Pileus 3–12 mm. wide, *persistently convex or campanulate,* varying subumbonate to subumbilicate, dry, hygrophanous, *white to pale fuscous or fuscous umber, pruinose with minute fuscous brown particles* scattered toward the white margin, crowded over the deeply colored disc, *striate:* margin slightly incurved at first, finally subcrenulate.

Stem 1–2.8 cm. × 0.5–0.8 mm., slightly thickened downward, hyaline pellucid, *viscid, often thickly glutinous toward the base, white, becoming pale fuscous then yellowish fuscous from the base* upward, the base abrupt and villous within the gluten.

Gills *broadly adnate to decurrent, subdistant,* 2–3 ranks, 9–14 primaries 1.3–3.5 mm. wide, *white* or pale fuscous at the base, often subcostate, with fuscous edge in the Brazilian specimens.

Flesh very thin, hygrophanous, concolorous: odor none.

Spores 5.7–10 × 3–4.7 μ, white, *luminous* when damp and fresh, pip-shaped oblong, aguttate, thin-walled, smooth, the contents cloudy, the wall amyloid.

Basidia 18–30 × 6.5–9 μ: sterigmata 4, 3.5–4 μ long.

Cheilocystidia 15–36 × 6–18 μ, forming a narrow sterile edge, clavate to subventricose or subglobose, often irregularly lobed but not digitate, thin-walled, smooth, with pale brown sap in the Brazilian specimens: pleurocystidia none.

Pileocystidia 25–65 × 15–40 μ, clavate-pyriform or subglobose, thin-walled, smooth, with deep umber sap, proliferating from the narrow stalk, set in clusters forming a disrupted palisade, more or less continuous over the center, the stalks 2–3 μ wide.

Caulocystidia –30 × 2–4 μ, as simple scattered subcylindric and irregular processes into the gluten from the narrow superficial hyphae of the stem, more abundant at the stem-apex and compacting into the hymenium: at the base of the stem, elongating into aseptate or sparsely septate hyphae –300 × 2–4 μ, tapering to slender points, projecting at right angles from the stem into the gluten, and with pale brownish ocher, slightly thickened, glutinous walls.

Hyphae in the stem longitudinal, clamped, the cells 100–400 × 8–22 μ, or – 35 μ wide at the base of the stem, up to 1100 μ long in the Brazilian specimens, the septa broad and transverse, without interweaving hyphae: colorless at first, but the walls thickening slightly and becoming pale brownish from the base upward.

Hyphae of the pileus as in the stem but with colorless walls: with a narrow pseudoparenchymatous layer, 1–3 cells thick, just below the palisade of pileocystidia, the cells 30–150 × 10–30 μ, with fuscous umber sap: in the gills, as in the pileus, not pseudoparenchymatous, the subhymenium c. 8 μ thick and composed of narrow hyphae 2–3.5 μ wide.

Hab. on dead leaves and twigs in the forest: tropical Asia (Ceylon, Malaya), Australasia (Rabaul), and America (Brazil).

This species can be found in Malaya every fungus season, but it is usually scattered and cannot be considered common. It is remarkable among luminescent fungi because only the fresh damp spores, as seen round the base of the stem on the stick or leaf, are luminous. Yet, I am unable to find any other distinctions from the well-known European and North American *M. rorida* than those given in the diagnosis, and they do not suggest a different species. For this reason, and because it is always advisable to check very carefully tropical records of temperate species, I have given a full description of the variety as I have found it myself in Malaya and Brazil.

Concerning the gills, Kühner gives 12–21 primaries and Smith 14–18 for typical *M. rorida.* Malayan specimens had 9–14 and the Brazilian 10–12.

Regarding the spores, there would seem to be a better distinction, but when analyzed in detail no qualitative difference appears. Thus, there are the following reliable records of the size and number of the spores and of the size of the basidium:

Spore-size μ	Number per basidium	Size of basidium, μ	Authority
13–14.5 × 6.6–7	2	—	Josserand, France
10–13 × 3.5–5.7	2	20–34 × 5–8	Kühner, France
10–15 × 4–5	2	30–36 × 5–6	Ricken, Germany
10.5–15 × 4–5.7	2	—	Corner, England
9–12 × 4.5–6	2	26–30 × 5–7	Smith, U.S.A.
8–10 × 4–5	4	—	Smith, U.S.A.
8–11 × 4	?	—	Rick, Brazil
7–10 × 3–4	?	—	Petch, Ceylon
8–10 × 4–5	4	23–30 × 7–8.3	Corner, Malaya
6.5–9 × 4–4.5	4	18–23 × 7–9	Corner, Malaya
5.7–8.5 × 3.5–4.7	4	18–24 × 6.5–8	Corner, Brazil

Now, on analyzing these figures in the way proposed in my paper on the connection between spore-size and number and basidium-size (**1948**), one finds the following relations:

For dispores,

Average spore-size 12.20 × 5.20 μ, volume 172.8 c.μ

Average basidium-size 29.3 × 6.0 μ, volume 575.1 c.μ

For tetraspores,

Average spore-size 7.95 × 4.28 μ, volume 76.25 c.μ
Average basidium-size 22.7 × 7.63 μ, volume 503.6.c.μ

The dispore has, thus, 2.27 times the volume of the tetraspore. Assuming a width of 4.28 μ for the tetraspore, a dispore-basidium should be able to produce four spores of the size 9.32× 4.28 μ, which is appreciably. longer than the observed average, though agreeing better with the maximum mean values of 9.0 × 4.5 μ found by Smith and myself for the tetraspores. However, the basidia of the dispores are larger than those of the tetraspores, apparently, having 1.14 times their volume. Allowing for this correction in calculating the volume of the tetraspores derivable from the dispore-basidium, one finds an average calculated tetraspore of size 7.89 × 4.25 μ, which agrees well with the observed average of 7.95 × 4.25 μ. Hence, I conclude that the 4-spored state of *M. rorida* differs from the 2-spored not only in this number of spores but in having slightly smaller basidia.

Regarding the luminescence, Dr. Haneda first recorded this in the specimens identified as *Omphalia* sp. from Rabaul (**1942**), and together we observed the same brilliant greenish luminescence of the fallen spores in Singapore. I surmise that the Ceylon specimens described by Petch as *M. rorida* and the Brazilian specimens found by myself in Rio de Janeiro and in Manaus had also this luminiscence, though I regret that, owing to the hasty conditions under which I managed to snatch a little time for mycology in Brazil, I forgot to check this point. It is not unlikely, indeed, that the temperate *M. rorida* itself may have luminous spores, for luminosity of various parts of the fruit-body is considered as authentically proved for some 15 species of *Mycena,* according to Wassink. On the other hand, *M. rorida* may have a luminous variety as *Panus stypticus.*

M. rorida is, thus, interesting from the distributional point of view. As a whole it appears to be cosmopolitan, mainly 2-spored in Europe, 2–4-spored in North America, and 4-spored in the tropics. In the viscid stem and the palisade of clavate cells on the pileus, making it truly pruinose, the species indicates a distinct section of the genus and, as I have shown in a forthcoming monograph on clavarioid fungi, such wide-spread distribution of sections, microgenera and, indeed, individual species seems to be the rule among basidiomycetes: the nearest allies of many temperate species are tropical, and *vice versa.*

SUMMARY

Dictyopanus luminescens, from Malaya, has wholly luminous fruit-bodies. Specifically it is characterized by the large spores 9–13 × 5–6 µ. The records of luminescence in *Polyporus rhipidium* from the Bonin Islands and from Australia may refer to *D. luminescens.*

Mycena rorida var. *lamprospora* has smaller spores, four per basidium, than the typical 2-spored state of the species, and these spores, when fresh and damp, are luminous. The variety is definitely recorded from Rabaul and Malaya, very probably from Ceylon and Brazil, and may be pantropical.

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PULLULARIA AS A CAUSE OF DETERIORATION OF PAINT AND PLASTIC SURFACES IN SOUTH FLORIDA [1, 2]

ERNEST S. REYNOLDS

(WITH 2 FIGURES)

GENERAL ECONOMIC SIGNIFICANCE OF PULLULARIA

In recent years *Pullularia* (**5**, **6**), usually under this generic designation or often under the older name, *Dematium pullulans* DeBary, has been reported with increasing frequency from various parts of this country and from other parts of the world. In the older references especially, it was often described as a saprophytic mold with no particular rôle assigned to it, but more recently it has been reported as an important if not a primary element of the sooty-molds growing on "honey-dew" in Europe (**30**, **45**) and New South Wales (**14**).

As an organism associated with deterioration of plant parts and plant products *Pullularia* has been reported sufficiently often to justify considering it of considerable economic significance. In some references it has been indicated as a cause or a cooperating cause of disease or injury in plants (**1**, **4**, **15**, **16**, **27**, **31**, **49**) or of damage to fruit (**10**, **11**, **12**, **13**, **46**). It appears in general to be "saprophytic or weakly parasitic" as stated by Bennett (**5**) in his studies of its effects on wheat.

As a flax retting organism it is reported from the Australian Commonwealth (**2**, **21**); and as an element of importance in the

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[1] Contribution No. 36, Moisture- and Fungus-Proofing Research Program, Bureau of Ordnance, Department of the Navy, and Contribution No. 40 from the Marine Laboratory, University of Miami.

[2] Presented at a joint session of the Microbiological Section of the Botanical Society of America and the Mycological Society of America, September 13, 1948. The opinions expressed herein are those of the author and not necessarily those of the organizations involved.

pulp and paper-mill mold floras it has been reported from Finland and Sweden (**22**, **25**, **28**, **39**), although it seems to have been reported only once as a possible member of the true paper-mold-flora (**47**, p. 289–290). As a timber blue-staining organism it is of frequent occurrence in Sweden (**7**, **8**, **22**, **37**) and Finland (**24**). Although listed as having been isolated from "cotton hairs" it is listed also as having no "cellulose decomposing power" (**47**, p. 255). A later study (**33**) describes a black deterioration of cellulose strips by *Pullularia* alone and also in association with some other molds; but recent studies at the Quartermaster Depot in Philadelphia find no proof of cellulose decomposition by various strains of *Pullularia* including some received from Florida (**3**, **36**, **50**).

In Puerto Rico *Pullularia* was isolated from human skin (**9**) as a possible cause of disease, but apparently no allergenic importance has been ascribed to it (**29**). Sheep leather has been found to be spotted by *Dematium* (**19**), which along with other molds has been listed by another writer as capable of altering the structure of wool (**35**). *Pullularia* has been reported in recent listings of molds obtained from military materiel exposed to contamination in tropical and sub-tropical areas (**3**, **50**) although no special rôle has apparently been assigned to it. We may conclude from the studies briefly reviewed above that *Pullularia* is of considerable general economic importance, especially in the paper-pulp industry and as a secondary or cooperating organism in injury to plants and to plant parts and products in various parts of the world, and that its importance is not confined to the sub-tropical and tropical areas.

As a significant cause of paint deterioration, *Pullularia* was originally reported in 1920 by Haenseler (**17**, **18**) to be found in association with other molds on painted surfaces in New Jersey. Since that time very little attention has been given to it either by practical paint men or by mycologists.[8] It was found in the course of my investigations that there was no reference to *Dematium* or *Pullularia* on paints under "Molds" or "Paints" or other pertinent headings in the Decennial Indexes of the Chemical Abstracts covering the years to and including 1936 and only twelve direct references down to that time to paint molds. During the last decade considerable activity has been shown in preparing and testing paints for resisting mold growth in general, but only occasional reference was made to *Dematium* as a paint mold. However, it appears from the most recent studies of Messrs. Goll and Coffey, and as reported in this paper, that the production of paint specifically resistant to *Pullularia* would be an important development.

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[8] After the preparation of this paper for presentation, a paper by Milton Goll and George Coffey entitled, "Mildew of Painted Surfaces," was published in the *Paint, Oil and Chemical Review* of August 5, 1948. These authors attribute a large part of mold deterioration of painted surfaces to *Pullularia.*

PULLULARIA IN SOUTH FLORIDA

The presence and possible importance of *Pullularia* in South Florida was first called to the attention of the writer through a series of paint samples in which the green, brown, and red painted surfaces had developed numerous dark, essentially black, points. Isolations from these spots produced either pure cultures of *Pullularia* or this mold was the only dematiaceous form present. Further study of these paint samples indicated that most of the black spotting which appeared like soot or black dirt particles actually was due to spots of *Pullularia* infection. A second series of paint panels exposed a few months later at the same site developed a similar heavy infestation of *Pullularia* on certain white paints (FIG. 1, A). Approximately a year later, a commercial testing agency called attention to a serious spotting of some plastic coated glass cloth samples (FIG. 1, *C, D*). These also proved to be heavily infested with *Pullularia* after only a few months of exposure. In all of these cases some other molds were present on the surfaces including *Cladosporium* and species of *Penicillium,* but the major spotting was due to *Pullularia.*

A more detailed study of each of these infestations and associated pure culture work has developed data which indicate possible explanations of some of the phenomena noted in the course of this study and also suggest further necessary studies in order to gain a satisfactory understanding of some of the problems involved.

PULLULARIA ON PAINTED SURFACES

As noted before, two paint tests have been made on which *Pullularia* has developed.[4] In the first series there were ten paint types, half of the samples of each containing anti-mold additive and half without. Half of the treated samples and half of the untreated were exposed vertically in a rack standing outdoors and the other half of each was in a "stabilized natural" test chamber (41) which approximated 30–35° C. and 80–85% relative humidity as averages for the higher ranges. The second paint series consisted of nine interior paint types having three specimens with anti-mold additive each and three untreated, two of each three exposed outdoors and one each in the test chamber. For about two months the outdoor exposures of the first series were in a mangrove swamp and then were transferred to an open, cemented platform near the bay. During part of the day this area was partly shaded from the direct sunshine, but was neither exposed to drip from vegetation nor near any large mass of vegetation.

During the first six to eight months of exposure there were no positive evidences of fungal growth on the outdoor panels of either series of paint tests. But six months later an undetermined dark specking of one of the dark colored outdoor panels of the first series had appeared. During the third six months period most of the dark colored outdoor samples of this series had this specking, which was especially abundant near the upper ends of the panels. Some of these brown to black dots were superficial on the paint film and some were somewhat imbedded in it. Petri-dish cultures on potato dextrose agar and Czapek's solution agar made from several of these specks on different panels gave *Pullularia* growth with no other dematiaceous mold developing from the inoculum used. In some cases brown mycelial webby growth on the paint film was associated with the dots. A microscopic examination of the dot type of infestations indicated that they were composed primarily of dark-brown, oval to globular, heavy-walled spores either single or in clumps of two to several spores. Except for this mold infestation the painted surfaces showed very little effect from "general weathering" and would have been considered in good condition. None of the white to ivory panels of the first series developed the *Pullularia* infestations. The panels in the test-chamber were also free from this infestation, although *Penicillium* was present on both treated and untreated samples of the green, brown, and red paint films. It was found, however, that the panels painted with the anti-mold treated paint developed only 5 to 10% of the number of *Penicillium* patches on the untreated paints.

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[4] These were originally set up, as planned by the paint department of the Centro Research Laboratories of Briarcliff Manor, N. Y., as a part of the general program of anti-mold toxicant studies.

In the second series of paint tests all of the outdoor specimens of two white paint types, including treated and untreated paints alike, developed a heavy infestation of dark brown to black specks which gradually changed the general appearance of the paint film from white to dull gray in about two years. Specimens of the dark globular bodies inoculated into agar plates all developed *Pullularia* growth centers. From figure 1, A & B, it can be seen that the typical panel (B) with mold growth has been definitely deteriorated as compared with a typical panel of another paint in the series (A) exposed similarly but not seriously attacked by the *Pullularia.* The magnified view shown in figure 1, E, demonstrates that the paint surface itself, showing among the mold spots, withstood the general weathering conditions well and although painted with an "inside" paint, would have been satisfactory if it had not become infested with *Pullularia.*

PULLULARIA ON PLASTIC PANELS

The plastic panels which were of commercial origin were glass cloth coated, as stated by the fabricator, with "Vinylite VYNV, having Dioctyl Phthalate as the main plasticizer but with tricresyl phosphate and some amounts of another plasticizer whose chemical composition is unknown to me." Metallic pigments of various colors were used and the totally inadequate amount of ten parts per million of pyridylmercuric stearate as anti-mold toxicant was used in certain coatings. The upper, infested surfaces of two panels, one representing the toxic-treated plastic (C) and the other not treated (D), are shown in figure 1, C & D. It was

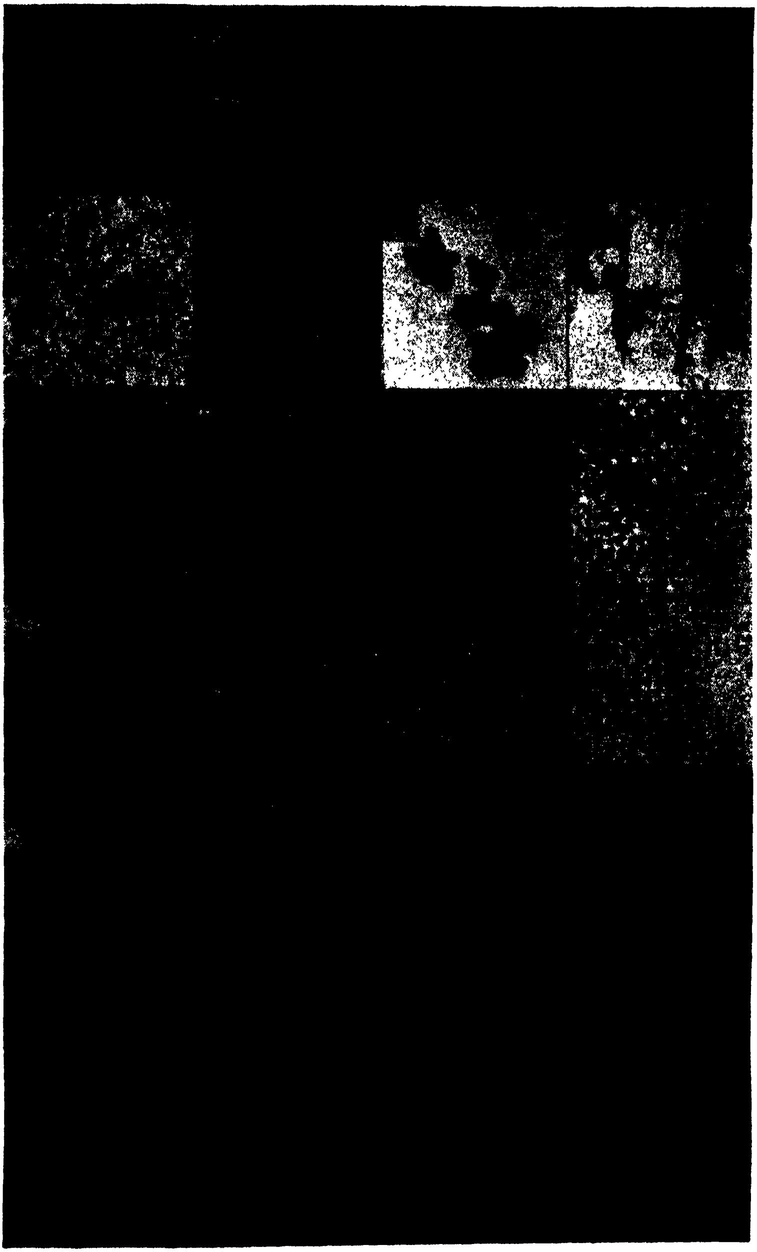

FIG. 1. *Pullularia* on painted and plastic surfaces.

evident from naked eye and microscopic examination that there was little difference between the amount of mold development on these two panels, as one would expect from the small amount of toxicant used, and that they had been well covered with the mold spots in the three months exposure period. A quantitative examination of a typical area on the non-toxic panel showed that out of a total of 62 mold colonies developed from a scraping, 53 were *Pullularia* and 9 were *Cladosporium*. An examination of ten of these plastic panels showed that all were rather heavily infested with this mold which was mainly of the black, spotted to granular type (FIG. 1, F), essentially identical with the main growth on the paint panels. In some cases the mold deterioration developed as vague, dark blotches up to four or five millimeters in diameter. On some panels the blotched condition was found also on the under side and on some, fairly large masses of the dark brown mycelium with various stages of chlamydospore formation were found on the under side where moisture could accumulate and remain for a longer period than on the upper surface, thus encouraging a more abundant vegetative growth. In still other cases a more even, very fine, black-dotted condition existed, but the microscopic appearances of the blotched areas and of this type were essentially alike (FIG. 1, G). Less frequently a mycelial web, similar to some spots on the paint panels, was intermixed with clusters of spores, on both upper and lower surfaces of some of the panels (FIG. 1, I). The majority of the hyphae in such cases had chains of chlamydospores in various stages of development (FIG. 1, H). These superficial differences seemed to be associated with differences in the plastic coating and probably with the atmospheric conditions at the time of development of the infestation. Collodion drops were placed on several spots on the upper surfaces of different panels and the partially dried film was mounted in lactophenol plus 10% O. T. as spreader. These microscopic mounts as seen in figure 1, G, H, I, give a very clear picture of the exact positional relationships of the mold on the plastic surfaces and of their detailed appearance. A microscopic examination of these plastic panels showed that while most of the spores were of the *Pullularia* irregular-globular type in small or large clusters, or less often in short chains, there were occasional stray spores of other types including *Pestalotia* and apparently *Cladosporium*[5] as the most frequent kinds. Very few dust granules were present on the upper surfaces where the black, granular infestation was most abundant.

Potato dextrose agar plates were inoculated in three to five places with mold material taken from the various types of growth described above and *Pullularia* developed in all cases. Occasional growths of other dark molds developed as well, especially *Pestalotia* and a dark mold not yet satisfactorily identified. *Trichoderma* developed on some of these plates, but did not originate from the inoculation centers except on one plate. It does not appear that any of these molds contributed largely, if at all, to the spotting of these plastic surfaces. General climatic deterioration of these plastic surfaces was noted in some cases, but the mold attacks were the primary cause of early deterioration.

The extent to which molds may penetrate and discolor resin-impregnated glass cloth is indicated in figure 1, J, K, taken from another series of tests on a different type of material, made in the high humidity chamber. The black, mixed mold growth penetrated among the strands of glass fibers to an undetermined but appreciable depth.

CULTURAL CHARACTERISTICS OF PULLULARIA

In petri-dish cultures on potato dextrose agar the *Pullularia* growth almost always starts out as a mucoid, milky, somewhat elevated, surface mass (FIG. 1, L), composed of the small, oval, colorless conidia or blastospores which multiply very rapidly for several days. Under favorable conditions this milky, mucoid growth can spread in four or five days to cover over a half or more of the agar surface in a 100 mm. petri dish culture. At this stage, but usually a little later, fine, radiating, colorless, submerged hyphae with attached conidia can be distinguished by a careful adjustment of lateral, reflected illumination against a black background under the higher powers of binocular magnification. This is especially well seen through the bottom of the petri dish. However, the great mass of conidia is produced by yeast-like budding of the conidia, which process, at a slightly later stage, gives rise to masses of these colorless conidia along the fine colorless hyphae, thus producing, near the center of the growth, broad, radiating bands of mucoid-milky growth tapering toward the distal ends of the hyphae and with frequent, tuberculoid enlargements where exceptionally rapid budding has taken place. This latter development takes place primarily if not entirely along the hyphae which radiate more deeply into the agar. In eight to ten days or earlier, a fringe of small, colorless hyphae usually more or less surrounds the mucoid to dark central area (FIG. 2, A), which may have also begun to pass into the next stage. At this time the whole culture may be entirely mucoid-milky in appearance, or fringed with hyphae, or there may be the beginning of the darkening associated with the production of brown and later black spores and hyphae (FIG. 2, B). This dark coloration may begin as a fine, scattered-dotted condition due to single scattered chlamydospores (FIG. 2, C). Further blackening is due either to dark walled hyphae (FIG. 2, A, D, E) changing rapidly to almost black and usually containing closely successive chlamydospores, or to the production of oval, brown spores similar in type to the colorless oval blastospores and forming broad bands of deep brown mucoid growth tapering toward the distal ends of the hyphae and with frequent tuberculoid enlargements (FIG. 2, D, E). In the still older cultures the entire growth may form a solid black mass including the submerged and surface layers (FIG. 2, F). The dark spores may vary from smooth to warty and may be single, in pairs, or in clumps of three to several (FIG. 1, G; FIG. 2, C, G). When formed within the hypha they may be single or from two to numerous and contiguous, or

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[5] Earlier investigators, as for example Laurent (23) and Massee (26), probably were confused by the fact that frequently *Pullularia* (then designated *Dematium pullulans*) produces smooth, oval, dark, two-celled spores which are very similar to spores of *Cladosporium*, and they identified these fungi as stages of the same mold. However, quoting from Bennett (5) "there is much evidence that Laurent's view is erroneous." Many plate cultures of *Pullularia* and of *Cladosporium* made in the course of the studies reported here clearly indicate, in agreement with Loew, Janczewski, Planchon, Hoggan, Brooks and Hansford and Bennett, as reported by Bennett (5), that there is no genetic relationship between the two, although frequently found growing together.

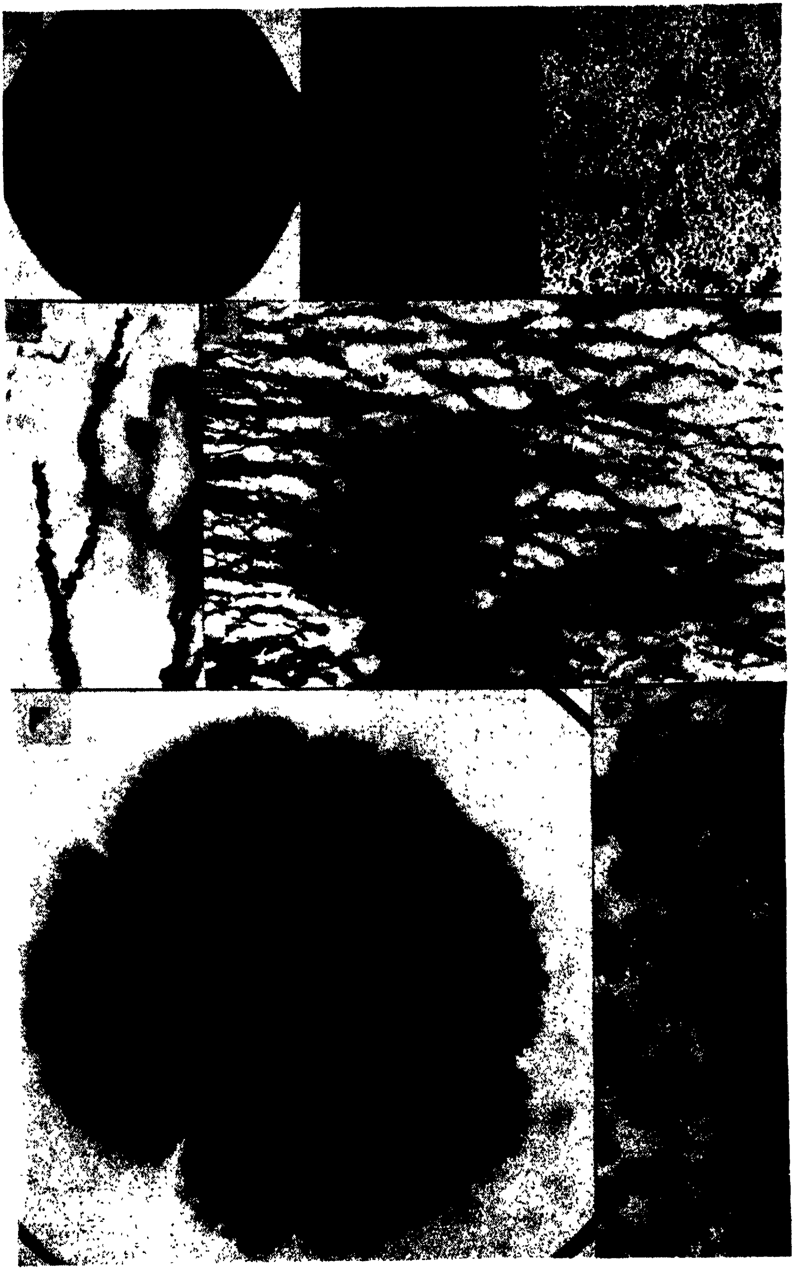

FIG. 2. *Pullularia* in culture.

sometimes with alternating groups of light colored vegetative cells of variable number between the groups of dark spores. It has been noted that a rapid blackening of the cultures occurs whenever somewhat unfavorable conditions exist, including competition with other molds on the same plate and drier conditions near the edge of the plate or where a thin agar layer is present. This reaction may be associated with the ability to resist unfavorable natural conditions such as those on the paint and plastic panels.

DISCUSSION

Resistant Nature of Pullularia. From a tabulation of reports on the occurrence of *Pullularia,* it appears that this fungus may be found especially abundantly in the sub-tropical, humid areas of the world but also frequently in the temperate, humid regions. However, its abundance on the upper, plastic surfaces, as viable inoculum, exposed facing the main source of heat and light in the Miami area, indicates that it is, under these conditions, highly resistant to high temperature, dryness and strong sunlight and can develop spores and hyphae (FIG. 1, G & H) during the brief periods of moisture, especially dew, characteristic of this area. On the other hand, Rennerfelt (38) reports that *Pullularia* grows best at about 27° C. on the synthetic medium which he used. It is not certain just how the dark, heavy-walled spores are produced on the paint and plastic surfaces. They may result from a very sparse mycelial development changing almost immediately into the heavy-walled chlamydospore stage, as seen in figure 1, H. Microscopic evidence from culture work indicates that this process can occur very early under unfavorable conditions and the fact that these specks on the paint and plastic surfaces are composed of small clumps and short chains of spores indicates that such mycelium as is formed rapidly attains the chlamydospore stage. Two physiological characteristics of *Pullularia* which may help to account for its ability to develop on paint and plastic surfaces outdoors are its dark colored spores, which are reported to be relatively more resistant to ultra-violet radiation than light colored spores (**13, 20, 44**), and its reported ability to assimilate elemental nitrogen (**43**). In addition to the early isolations of *Pullularia* from the paint surfaces, later tests (August to November 1948) gave rise to colonies on agar plates in all cases, indicating long continued viability of the spores under these conditions.

Dissemination of Pullularia. Since the distribution of *Pullularia* infection spots on the paint and plastic surfaces is fairly uniform over the areas affected, it points to the probable spread of the spores by air currents rather than by animal agencies or rain. The fact that the heaviest infestation on the long paint panels was on the upper portions substantiates this view as does also the fact that the infestations were not in lines or in short trails such as one finds when fungi are distributed by crawling insects.

The source of the inoculum for these infestations is at present unknown since the paint panels and the plastic panels were both in open exposure sites, but at a distance of several miles from one another. In an informal report it has been stated to the author that in the course of allergenic studies being carried on in Miami, *Pullularia* has not been recognized as a common mold in the types caught on exposed agar plates. It has not been possible as yet for the writer to carry out tests of this type. The fact that neither of the paint tests showed any serious infestation until the second year of exposure indicates that there may have been very little *Pullularia* distribution during the first year (1946), and considerable during the second year of exposure (1947), or growth conditions were very different throughout these two years. The plastic samples were placed on test in January 1948 and had acquired a rather heavy infestation when the photograph (FIG. 1, C, D) was taken in April. Hence, the early part of that year was favorable for *Pullularia* distribution and development. In the Central and Southwestern United States, it is reported (**29**) that *Pullularia* may occur as a "shower" "and make up a significant fraction of the total mold count" of air-borne molds, although it is not listed by these authors as an allergenic mold. *Pullularia* has been reported as a soil inhabitant (**32, 42, 48**), but there seem to be no published data of its multiplication in the soil, or of any great abundance in that medium. In the case of the paint tests, at least, it appears improbable that the heavy infestation could have been from adjacent soil sources, since these tests were on exposure near the shore of Biscayne Bay and not near any source of open soil. Moreover, there was very little evidence of soil particles on the paint samples and the heaviest infestation was at the upper ends of the panels. From these data it appears that although this mold is not uniformly an air constituent it may from time to time be carried in considerable amounts, possibly for considerable distances. Experience in Europe (8, 40, 45) has shown also that the presence of *Pullularia* as spores in the air and as sooty-mold is spasmodic or seasonal, but the sources of these spores do not appear to have been determined. The source and type of inoculum are still problematical where this mold develops in outdoor locations. It is possible, although by no means certain, that each small spot of infestation, on these paint and plastic panels, came from an original air-borne inoculum. However, it was clear from an examination of the plastic panels that some growth had taken place at many of the centers of infestation.

A variable ability of different cultures of *Pullularia* to produce the black pigment and numerous chlamydospores has been noted, but no specific studies on this have been made by the writer. It is stated by one investigator (37) that this organism may be able to synthesize to some extent the substances necessary for growth, but not for spores and color of mycelium.

SUMMARY AND CONCLUSIONS

The evidence presented in the current studies shows that this mold may be responsible for serious deterioration of painted surfaces in southern Florida quite aside from and prior to general climatic injury and is therefore an important subject of study for methods of prevention in order to prolong the early life of an otherwise satisfactory paint.

It is also demonstrated that certain plastic surfaces are likewise subject to serious deterioration by this same organism either associated with or prior to general climatic injury. The extent to which different types of plastics and plastic surfaces are subject to this type of deterioration should be given separate and careful consideration in the attempt to provide satisfactory plastic materials for use in the areas where this mold is prevalent.

The ready recognition of *Pullularia* as an organism causing injury or deterioration depends upon a knowledge of its characteristic appearance and stages under varying conditions, since, as indicated above, it is able to adapt itself readily to conditions which in general are unfavorable to most molds. Data and observations are included to provide some of this information. Further studies are in progress to round out the knowledge of its developmental stages under adverse conditions and the source and dissemination of the inoculum for the heavy infestations which occur in the open.

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EXPLANATION OF FIGURES

FIG. 1. Photomicrographs showing deterioration of paint and plastic surfaces due to *Pullularia* and other Dematiaceae. A. (1 ×) White painted panel showing heavy spotting with *Pullularia*. B. (1 ×) Panel painted with a different paint at the same time and exposed simultaneously with (A), but without *Pullularia* infestation. C. (⅕ ×) Plastic panel with a small amount of fungistat present. D. (⅕ ×) Untreated plastic panel, but both C and D heavily infested with *Pullularia* after three months' exposure. E. (8 ×) A portion of (A) showing various sizes of patches of the mold with the background of otherwise undeteriorated paint surface. F. (60 ×) A portion of (D) showing various sized patches of the mold. G (225 ×) Patches of *Pullularia* removed from a plastic panel by a collodion peel showing spore clumps of the "granular type" of growth. H. (225 ×) *Pullularia* in a collodion peel from a plastic panel showing detail of the "mycelial type" of growth. I. (60 ×) A portion of (D) showing patches of the mold variously connected by mycelial strands. J. (1 ×) A type of deterioration of another resin-impregnated glass cloth associated with an infestation of dematiaceous molds. K. (10 ×) Portion of (J) showing the elongated, black streaks of fungal growth following and intermixed with the glass fibers L. (8 ×) A very young "mucoid" growth of *Pullularia* on potato dextrose agar with a small black center. Note the radiating hyphae with large masses of milky blastospores forming the blunt irregular marginal points with the hyphal tips projecting.

FIG. 2. Photomicrographs showing certain characteristics of *Pullularia* on potato dextrose agar medium. A. (2 ×) A young growth, older than in figure 1, L, with a white, radiating margin of hyphae and black mature center with radiating dark hyphae which finally become amalgamated into a solid black mature growth. B. (1 ×) A later stage showing the light young marginal area composed of hyphae and closely packed blastospores and the dark, mature central area. C. (225 ×) A very young growth removed and slightly flattened under a cover glass showing the masses of colorless blastospores and scattered dark, chlamydospores developed directly in the blastospore "mucoid" mass with no hyphal development. D. (30 ×) Dark hyphae with numerous scattered "tuberculoid" masses of dark blastospore-like spores as seen through the petri dish bottom against a light background largely composed of blastospores together with fine hyaline hyphae. E. (60 ×) A portion of (D). Colorless "tuberculoid" hyphae of otherwise similar type are developed in the younger stages of growth and may be found in the same petri dish culture with the dark ones shown here. F. (1 ×) A mature culture in which essentially all of the hyphae and spores are deep brown to black and with heavy walls. G. (225 ×) Short chains and clumps of dark chlamydospores with numerous colorless blastospores forming the background. A later stage than shown (FIG. 2, C).

NOTES AND BRIEF ARTICLES

NOTICE OF FORAY

The Annual Foray will be held August 7 to 11 at the University of Wyoming Science Camp, located in the Medicine Bow Mountains, forty miles west of Laramie at an elevation of approximately 9,500 feet. Within a radius of ten miles from the camp, the elevation ranges from 7,800 to 12,000 feet. The camp location offers an excellent opportunity to study the mycological flora of plant associations representing alpine, subalpine, montane, foothills, and plains zones. Laboratory accommodations and equipment (microscopes, presses, driers, etc.) will be available. The University is reserving accommodations for forty members of the Mycological Society. Board and room will be provided at approximately $4.00 a day per person. Blankets, linens, towels, and soap will be furnished. Although the days are warm, the nights are cool; consequently, suitable warm clothing is essential. Mail to be delivered during the Foray should be addressed in care of the University of Wyoming Science Camp, Centennial, Wyoming. Communicate at the earliest possible date with Professor W. G. Solheim, University of Wyoming, Laramie if you plan to attend and list the names of individuals who are expected to accompany you.—KENNETH B. RAPER.

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THE GROWTH OF TRICHOPHYTON MENTAGROPHYTES AND FIVE OF ITS VARIANTS AS AFFECTED BY SEVERAL NITROGEN SOURCES

ILDA MCVEIGH AND FLORENCE CAMPBELL

(WITH 2 FIGURES)

The phenomenon of variation in the imperfect fungi has long been recognized. It is especially important because the identification of the various species depends largely on their appearance in culture, and failure to take cognizance of the variation displayed by most species in laboratory culture has led to a great deal of confusion in their taxonomy. Skinner, Emmons, and Tsuchiya (4) estimate that the number of true species can perhaps be reduced to one tenth of the reported number by a critical comparison of strains and a proper evaluation of variability.

Like many other imperfect fungi, *Trichophyton mentagrophytes* gives rise to pleomorphic variants as the culture increases in age. These can be distinguished by rate of growth, morphology of colony, pigment production, microscopic appearance, and physiological characteristics. Nine such variants were isolated by Robbins and McVeigh (3) and have been maintained in culture apparently unchanged since their isolation.

Little work has been done on the nutrition of variants of imperfect fungi from a comparative standpoint. Robbins and Ma (1)

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and Robbins and McVeigh (2) included a few variants of *T. mentagrophytes* in their nutritional investigation of that fungus. These are, apparently, the only examples of such comparative studies. The purpose of this investigation was to determine the comparative degree to which various forms of *T. mentagrophytes* are able to use the same sources of nitrogen as well as the ability of any one form to utilize different sources of nitrogen.

MATERIALS AND METHODS

The strains of *T. mentagrophytes* used in this study were obtained through the courtesy of Dr. William J. Robbins, New York Botanical Garden, New York City. The normal form (N) was isolated from a human subject in 1942, and has been maintained in laboratory culture apparently in its original condition since that time. In addition to this form, the nine variants isolated from it by Robbins and McVeigh (3) were also included in the preliminary work. These investigators designated these variants B, A, 4a15, 7a14, 7a38, 6a24, 2a37, and D1. On the basis of preliminary experiments six of the forms which exhibited wide differences were selected for subsequent experiments.

They were maintained in stock cultures, apparently unchanged, by weekly transfers to fresh agar slants. The stock medium had the following composition:

Dextrose	50.0 g.
KH_2PO_4	1.5 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.
Thiamin hydrochloride	1500.0 μ-moles
Neopeptone	2.0 g.
Distilled water	1000.0 ml.
Agar	15.0 g.

To this medium the following mineral supplements were added in parts per million: B, 0.005; Mn, 0.01; Zn, 0.09; Cu, 0.02; Mo, 0.01; and Fe, 0.01. The pH of the medium ranged between 5.0 and 5.5.

The basal medium was the same as the stock medium indicated above except for the nitrogen source, which was varied, and the agar. The agar was purified according to the method described by Robbins and Ma (1). The nitrogen sources were of chemically pure grade, and, except for urea, were added to the basal medium in quantities containing equivalent amounts of nitrogen, using 2 g./l. of asparagine as the basis of comparison. For the amino acids, calculations were based on the nitrogen content of the biologically active form. The nitrogen sources used included potassium nitrate, ammonium sulfate, neopeptone, casein hydrolysate, urea, asparagine, glutamic acid, tyrosine, phenylalanine, arginine, and leucine.[1]

Both liquid and agar media were used. The agar media were added in amounts of 15 ml. to 20 × 180 mm. test tubes. After autoclaving at a temperature of 121.5° C. for 15 minutes, the tubes were slanted as uniformly as possible and the media allowed to solidify. When liquid media were used amounts of 15 or 25 ml., depending upon the experiment, were added to 125 ml. Erlenmeyer flasks. These were sterilized as described above.

All inoculations were made from cultures 5 to 7 days old, by adding uniform portions of mycelium and spores about the size of a pin head to the center of the agar slant. Larger bits of inocula were used for the liquid cultures because the organism grows more slowly on liquid media. All tests were made in triplicate, and the average results calculated. The experimental as well as the stock cultures were incubated at 30° C.

All glassware was cleaned with detergent and then rinsed seven times with tap water and twice with distilled water before using. The chemicals were of C.P. grade.

Results from the cultures grown on agar slants were obtained by measuring the diameters of the colonies at intervals of 3, 7, 10, and 14 days and also by observing thickness of the mats. At the end of the incubation period, the hydrogen ion concentrations were determined approximately by means of pHydrion paper. In some experiments, supplementary results were obtained by determining the weights of the dried mycelia. This was done by autoclaving

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[1] On the basis of the amount of nitrogen in 2.0 grams of asparagine, the nitrogen sources were added per liter of basal medium in the following amounts: casein hydrolysate, 2.71 g.; neopeptone, 2.91 g.; ammonium sulfate, 2.0 g.; l-glutamic acid, 4.38 g.; l-tyrosine, 5.49 g.; l-leucine, 3.96 g.; l-arginine monohydrochloride, 1.59 g.; dl-phenylalanine, 10.0 g. Urea was added to the basal medium in amounts of 1.8 g./l. The nitrogen contained in this amount of urea was approximately twice that in the media in which other compounds supplied the nitrogen.

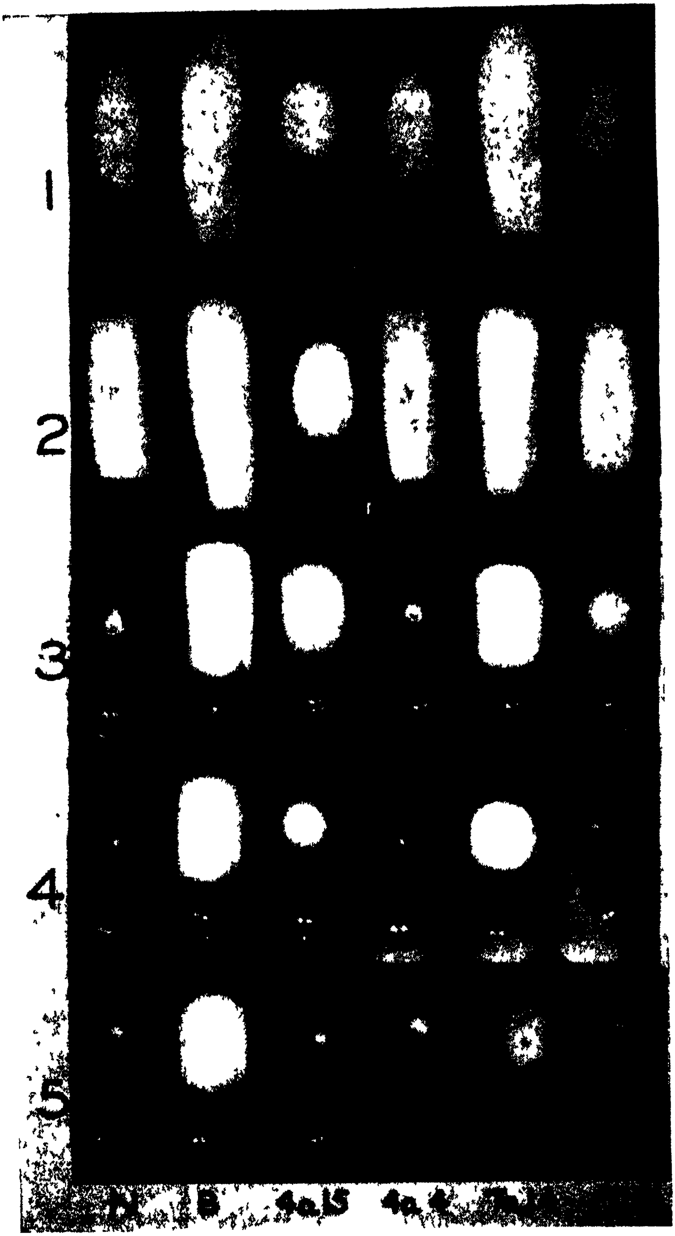

Figure 1. Normal and pleomorphic forms of *T. mentagrophytes* grown on basal agar medium containing: 1. Neopeptone—age, 14 days; 2. Casein hydrolysate—age, 14 days; 3. Asparagine—age, 10 days; 4. Urea—age, 10 days; 5. Ammonium sulfate—age, 10 days.

the cultures, removing the mats from the melted agar, washing them with hot distilled water, and drying them at 100° C. for 24 hours. The mats were placed in a desiccator over drierite until they cooled to room temperature, and were then weighed.

Dry weights and hydrogen ion determinations were also made of the cultures grown in liquid media. After an incubation period of 14 days the hydrogen ion concentrations were determined with the pHydrion paper, and the cultures autoclaved. They were then filtered through tared Gooch crucibles containing filter paper discs. The mats were washed thoroughly with distilled water and after drying, weights were determined as indicated above.

RESULTS

A preliminary experiment was designed to give an indication of the relative ability of the ten forms of *T. mentagrophytes* to use various sources of nitrogen, and to enable the selection, for further investigation, of those variants which exhibited the greatest differences. Five sources of nitrogen (casein hydrolysate, neopeptone, asparagine, potassium nitrate, and ammonium sulfate) were used. These were added separately to portions of the basal medium in concentrations of 2 g./liter.

The results of this experiment indicated that the best growth of all forms of this organism was obtained when a complex organic nitrogen source, such as casein hydrolysate or peptone, was used. Moderate growth was obtained on the medium containing asparagine, whereas the nitrogen of the inorganic compounds was almost completely unavailable, especially to the conidial forms. Ammonium nitrogen, in the form of $(NH_4)_2SO_4$, was somewhat more beneficial to the fast growing or mycelial forms than nitrate nitrogen (KNO_3). Growth on potassium nitrate was little, if any, better than that which could be accounted for by the nitrogen carried over with the inoculum.

Differences in growth characteristics of the various forms were decidedly less distinct on the media containing the complex nitrogen sources, peptone and casein hydrolysate than on asparagine, although the various forms were still distinguishable. Differences were most obvious on the medium containing asparagine, and were somewhat less apparent on that containing ammonium sulfate.

With potassium nitrate as the only source of nitrogen, growth of all forms was so poor that they were indistinguishable.

Those forms which varied from one another most in amount of growth and in appearance were selected for further study. These were the normal form N and five of its variants—B, 4a15, 4a4, 7a14 and D1. Three of these forms tended to be mycelial in character (B, 7a14 and 4a15) and three tended to be conidial (N, 4a4 and D1). The normal form and 4a4 are much alike. Variant D1 produces the least mycelium of any of the isolates. Of the three mycelial forms, 7a14 and 4a15 were considered intermediate between B, which produces abundant mycelia, and the conidial forms (N, 4a4 and D1), which produce much less mycelia and many conidia.

In the second experiment five nitrogen sources (neopeptone, casein hydrolysate, asparagine, urea, and ammonium sulfate) were used. Except for urea, these were added to the basal medium in quantities containing equivalent amounts of nitrogen. Urea was added in amounts of 1.8 g./l. which provided approximately twice the amount of nitrogen supplied by the other sources. Because growth after three days was so slight and because of the possibility of pleomorphisms being present in 10 days, measurements of colony diameters taken after 7 days of incubation were considered to be the most accurate.

Table I summarizes the measurements of 7-day cultures on the five nitrogen sources; figure 1 includes photographs taken of 10-day

TABLE I

Average Colony Diameters of Triplicate Cultures of the Normal Form of *T. mentagrophytes* and Five of Its Variants When Grown on the Basal Agar Medium with Five Different Sources of Nitrogen. Age: 7 Days

Form	Nitrogen source				
	Neopeptone	Casein hydrolysate	Asparagine	Urea	Ammonium sulfate
	mm.	mm.	mm.	mm.	mm.
B	44.7	44.7	21.5	23.0	21.0
7a14	41.3	35.0	19.7	16.8	18.5
4a15	23.0	18.0	15.5	16.0	9.3
4a4	39.7	36.7	14.0	16.7	16.3
N	41.3	33.7	13.7	15.0	14.3
D1	27.3	22.0	6.0	8.7	12.7

cultures on asparagine, urea and ammonium sulfate and 14-day cultures on neopeptone and casein hydrolysate. On the basis of general appearance, growth of each of the forms on the five sources of nitrogen was in the following order: neopeptone = > casein hydrolysate > asparagine > urea = > ammonium sulfate. The measurements of the diameters of the colonies are not always an indication of amount of growth. A colony may be widespread but composed of a very sparse mycelium while others of small diameters may be composed of a thick, compact mycelium. As mentioned previously, the differences between the forms were less distinct on the media containing complex nitrogen sources than on those containing simpler ones.

Of the six forms, B grew most luxuriantly on each of the sources of nitrogen supplied. It produced dense, fluffy, highly mycelial colonies on each of the media; the density and extent of growth were nearly equal on neopeptone and casein hydrolysate, whereas considerably less growth occurred on the simpler nitrogen sources. Variant 7a14 closely resembled B, but was less spreading and usually somewhat less dense. On ammonium sulfate 7a14 was quite different from B in that it formed a decidedly thinner, flatter mycelium. Variant B formed no pigment in any of the media, while 7a14 produced a pale, gold pigment when grown on the medium which contained casein hydrolysate.

Variant 4a15 ranked third in amount of growth although, on the basis of measurements of colony diameters, it sometimes appeared to have produced less growth than 4a4 or N. Growth was less widespread on peptone, casein hydrolysate and ammonium sulfate, but the colonies were denser and more mycelial in character than those of N and 4a4. On neopeptone and casein hydrolysate dense, moderately fluffy colonies with cream colored mycelia developed. Growth on the simpler nitrogen sources was thinner and less widespread, especially that on ammonium sulfate. A pale gold pigment was produced by this form on two types of media, namely those containing neopeptone and urea.

Variant 4a4 was almost indistinguishable from the normal form (N) on all media. The slight differences between the two in amount of growth were probably no more than could be accounted for by experimental error. Both were very powdery on neopep-

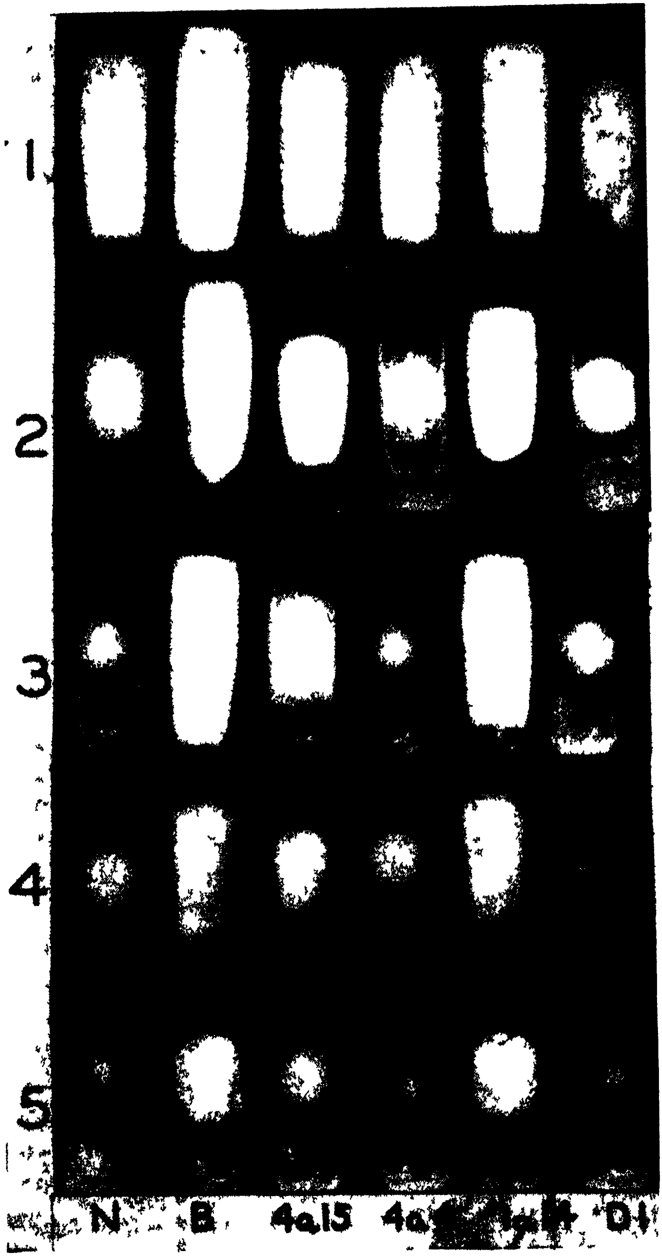

FIGURE 2 Normal and pleomorphic forms of *T. mentagrophytes* grown for 14 days on basal agar medium containing: 1. Casein hydrolysate, 2. Glutamic acid; 3. Arginine, 4 Tyrosine; 5. Leucine.

tone and casein hydrolysate, producing a dense subsurface mycelium with a flat mat of aerial hyphae. A brown pigment was produced by both forms on these media. On asparagine, a thin mycelium that was largely subsurface was produced. The colonies were very powdery and a deep gold-brown pigment developed. Growth on urea and ammonium sulfate was very scant, although widespread.

Variant D1 was the most slow growing of the cultures on all the media. It grew poorly on urea and ammonium sulfate and produced a very thin, but widespread mycelium; on asparagine its growth was powdery with a thin mycelium, and a gold brown pigment was produced. Colonies on casein hydrolysate had wrinkled centers after 10 days of incubation. On neopeptone the colonies were thinner, not wrinkled, and very powdery.

TABLE II

HYDROGEN ION CONCENTRATIONS OF CULTURE MEDIA CONTAINING FIVE DIFFERENT NITROGEN SOURCES ON WHICH THE NORMAL FORM OF *T. mentagrophytes* AND FIVE OF ITS VARIANTS HAD BEEN GROWN FOR 14 DAYS

Form	Nitrogen source				
	Neopeptone	Casein hydrolysate	Asparagine	Urea	Ammonium sulfate
	pH	pH	pH	pH	pH
B	5.0–5.5	5.0–5.5	5.0–5.5	5.0–5.5	5.0–5.5
7a14	5.0–5.5	5.0–5.5	5.0–5.5	5.0–5.5	5.0–5.5
4a15	5.0–5.5	5.0–5.5	5.0–5.5	5.5–6.0	5.0–5.5
4a4	5.0–5.5	6.5–7.0	7.5–8.0	7.5–8.0	5.0–5.5
N	5.0–5.5	6.5–7.0	7.5–8.0	7.5–8.0	5.0–5.5
D1	5.0–5.5	6.5–7.0	7.5–8.0	7.5–8.0	6.5–7.0

The hydrogen ion concentrations of the culture media were determined after 14 days of incubation to discover whether or not any change in pH could be correlated with the type ot growth. The determinations are summarized in table II.

The results given in table II indicated a correlation between the type of growth of the different forms and the changes produced in the hydrogen-ion concentrations of the culture media. In the presence of neopeptone, a natural buffer, no changes in pH occurred in the presence of any of the forms. In media containing casein hydrolysate, asparagine, and urea, the slow-growing, conidial forms

(4a4, N, and D1) tended to make the culture media more alkaline, whereas the mycelial forms (B, 7a14 and 4a15) caused little or no change. All culture media had been adjusted to a pH of 5.0–5.5 before inoculation.

To substantiate the results obtained on agar slants the six forms of *T. mentagrophytes* were grown in liquid media using casein hydrolysate, asparagine, and ammonium sulfate as the sources of nitrogen. The weights of dried mats from 14-day cultures are summarized in table III. Also included in this table are calculations of growth percentages of the five variants based on the growth of the normal form (N) as 100 per cent. These are designed to give an indication of the relative ability of the variants to utilize the various nitrogen sources as compared with that of the normal form.

TABLE III

AVERAGE DRY WEIGHT OF TRIPLICATE CULTURES OF SIX FORMS OF *T. mentagrophytes* ON A BASAL LIQUID MEDIUM CONTAINING THREE DIFFERENT NITROGEN SOURCES. AGE, 14 DAYS. Included are calculations of percentages of growth based on that of the normal form

Form	Nitrogen source					
	Casein hydrolysate		Asparagine		Ammonium sulfate	
	mg.	%	mg.	%	mg.	%
B	188.3	285	88.1	2447	0.73	561
7a14	176.8	266	20.3	564	0.33	254
4a15	131.8	199	4.2	117	0.33	254
4a4	48.1	73	3.3	92	0.13	100
N	66.1	100	3.6	100	0.13	100
D1	36.4	55	0.9	25	0.03	23

Results obtained from cultures grown in liquid media were not as uniform as those from agar cultures, probably because, in some instances, the inocula became submerged and did not grow as rapidly as those which remained on the surface. However, growth on each of the sources of nitrogen was approximately of the same order in the liquid as in the agar medium. The best growth of all forms was obtained with casein hydrolysate as the source of nitrogen, while asparagine gave fair growth and ammonium sulfate yielded poor growth. The relative magnitude of growth of the six forms also remained the same, although in the medium containing casein hydrolysate the differences between N and 4a4 were greater than was expected. This, however, might be explained by the fact that the inoculum in one or more of the flasks of 4a4 had become submerged.

The hydrogen ion concentrations of the cultures grown in liquid media were determined after 14 days of incubation. These are summarized in table IV. The same relationship between the character of growth and the changes in pH was found to exist here as was present in the agar cultures. Again the slow-growing conidial forms caused the culture media to become more alkaline than it was at the time of inoculation, while the mycelial forms caused little or no change except on the asparagine medium, and on this the change was less than that produced by the conidial forms.

TABLE IV

HYDROGEN ION CONCENTRATIONS OF LIQUID CULTURE MEDIA CONTAINING THREE DIFFERENT NITROGEN SOURCES ON WHICH THE NORMAL FORM OF *T. mentagrophytes* AND FIVE OF ITS VARIANTS HAD BEEN GROWN FOR 14 DAYS

Form	Nitrogen source		
	Casein hydrolysate	Asparagine	Ammonium sulfate
	pH	pH	pH
B	5.0–5.5	5.5–6.0	5.0–5.5
7a14	5.0–5.5	6.5–7.0	5.0–5.5
7a15	5.0–5.5	5.0–5.5	5.0–5.5
4a4	6.5–7.0	6.5–7.0	5.5–6.0
N	6.5–7.0	6.5–7.0	5.0–5.5
D1	6.5–7.0	7.5–8.0	6.0–6.5

The remaining experiments were designed to determine the ability of the six forms of *T. mentagrophytes* to use each of the following amino acids as a source of nitrogen: glutamic acid, tyrosine, arginine, phenylalanine, and leucine. Each of these had been reported by Robbins and Ma (1) to support growth of the normal form. Growth on casein hydrolysate was used as the standard for comparison. All nitrogen sources were used in quantities containing equivalent amounts of nitrogen.

Average diameters of the colonies of 7-day cultures of each of the six forms of *T. mentagrophytes* grown on the various amino acids and casein hydrolysate are given in table V; figure 2 shows photographs of 14-day cultures on the same media. No single amino acid yielded growth comparable to that obtained on casein hydrolysate. Judging by general appearances, growth of each of the forms on the amino acids was in the following order: glutamic acid > arginine > tyrosine > leucine. None of the forms grew on phenylalanine except B and 7a14, of which only one transfer out of three of each grew.

Growth on glutamic acid most nearly approached that on casein hydrolysate, especially that of the mycelial forms. Variants B, 7a14 and 4a15 each formed very dense, fluffy, uniform colonies which differed from each other principally in the extent of growth. Variant 7a14, however, was unusual in that it produced very large amounts of bright gold pigment.

TABLE V

AVERAGE COLONY DIAMETERS OF TRIPLICATE CULTURES OF SIX FORMS OF *T. mentagrophytes* ON BASAL AGAR MEDIUM CONTAINING FIVE DIFFERENT NITROGEN SOURCES. AGE, 7 DAYS

Form	Nitrogen source				
	Casein hydrolysate	Glutamic acid	Arginine	Tyrosine	Leucine
	mm.	mm.	mm.	mm.	mm.
B	43.5	39.3	35.0	24.7	19.0
7a14	35.7	30.3	31.3	21.5	14.7
4a15	37.0	26.3	29.3	18.3	15.3
4a4	35.5	18.3	15.7	10.3	14.3
N	36.0	20.7	16.7	12.0	15.0
D1	24.0	14.7	15.0	3.7	7.7

These three variants also grew well with arginine as the nitrogen source, although the colonies of 4a15 were less dense than those of B or 7a14. On tyrosine they formed very dense fluffy mycelia which appeared to have grown in concentric rings. Colonies of B and 7a14 on leucine were dense but not uniform in elevation and not clearly defined at the edges. The mycelium of 4a15 on this medium was flat and somewhat less dense than that of B or 7a14.

The individual amino acids were less available to the slow-growing, conidial forms than to the more rapidly growing, mycelial ones. Growth of N, 4a4 and D1 on glutamic acid, the amino acid which yielded best growth of these forms, fell far below that on casein hydrolysate. When this acid was the source of nitrogen, the normal form (N) and variant 4a4 developed a moderately dense mycelium which was almost entirely below the surface of the agar. These colonies had a coarse powdery appearance. Variant D1 was less spreading than N or 4a4, but formed somewhat more aerial growth.

Although the diameters of the colonies of N and 4a4 were larger on arginine than on tyrosine after 7 days of incubation, the mycelia were decidedly more dense on the latter; and as the cultures aged, the diameters of colonies on tyrosine became nearly equal to those on arginine. Colonies became powdery on both amino acids, but pigment was produced only when arginine was the source of nitrogen. Growth of variant D1 on arginine was definitely superior to that on tyrosine. On the former, it developed a fairly dense subsurface mycelium with some thin, flat aerial growth. It became powdery and formed yellow-brown pigment in the medium. When tyrosine was the nitrogen source, D1 formed a moderately dense aerial and subsurface mycelium that spread very little.

Growth of the three conidial forms on leucine was poor. The normal form and variant 4a4 developed a thin mycelium that was almost entirely subsurface. The colonies had a very coarse powdery appearance and produced a yellow-brown pigment. Variant D1 formed small powdery colonies whose mycelia were largely subsurface.

To supplement the results obtained by observation and by measurements of the diameters of the colonies, dry weights of mats of 14-day cultures grown in liquid and in agar media were determined. These weights are given in table VI.

Results obtained from liquid and agar media were essentially the same, although weights of mats grown on agar media tended to be higher than those from liquid media. This was especially true for the slow-growing conidial forms.

The order of growth of the three mycelial forms (B, 7a14 and 4a15) on the five nitrogen sources as determined by dry weight was as follows on both the liquid and agar media: casein hydrolysate > glutamic acid > arginine > tyrosine > leucine. Mats grown in the liquid medium with tyrosine as the nitrogen source weighed less than was expected on the basis of the weight of those on the agar medium. This may have been because the tyrosine precipitated out of the medium after autoclaving and held the inoculum under the surface of the liquid. Growth of 4a15 on arginine in the agar medium was slightly less than that on tyrosine but in the liquid medium containing arginine the mats weighed seven times those on tyrosine. The weights of dried mats of cultures which had been grown on casein hydrolysate were less in this instance than were those previously given (TABLE III); only 15 ml. of medium were used per flask in this experiment, whereas 25 ml. had been used per flask in the previous one.

TABLE VI

AVERAGE DRY WEIGHTS OF TRIPLICATE CULTURES OF SIX FORMS OF *T. mentagrophytes* GROWN FOR 14 DAYS ON AGAR AND ON LIQUID MEDIA CONTAINING FIVE DIFFERENT SOURCES OF NITROGEN

Form	Nitrogen source									
	Casein hydrolysate		Glutamic acid		Arginine		Tyrosine		Leucine	
	Liquid	Agar	Liquid	Agar	Liquid	Agar	Liquid	Agar	Liquid	Agar
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
B	164.7	153.9	100.9	109.5	47.7	78.2	15.1	78.2	15.5	50.4
7a14	138.0	133.2	85.7	105.1	23.1	68.9	14.4	45.1	4.3	35.7
4a15	106.7	97.6	66.7	76.5	14.7	30.7	2.1	39.9	1.3	8.9
4a4	38.6	54.5	1.7	15.4	3.5	5.9	1.5	9.6	1.0	1.3
N	30.1	53.8	1.7	16.2	1.4	5.0	1.2	10.2	1.6	1.2
D1	26.8	40.0	2.6	13.5	0.2	4.3	0.3	1.5	1.3	1.6

The dry weights of the mats of the conidial forms were so small that comparisons of yields on the various sources of nitrogen are not significant. These forms produced much heavier mats on casein hydrolysate than on any one of the amino acids. It might also be pointed out that mats of these forms grown on the agar medium containing glutamic acid were significantly greater than those on the same basal medium with arginine, tyrosine or leucine serving as the source of nitrogen.

The order of magnitude of growth of the six forms was essentially the same as that found in previous experiments, that is, B grew best on the media and D1 poorest while the others were intermediate. Some small variations in order were found among the slow-growing conidial forms; however, the mat weights of these when grown in certain media were so small that experimental errors in weighing could account for these differences.

The hydrogen ion concentrations of the cultures grown in liquid and in agar media were determined after 14 days of incubation. These values are given in table VII.

No change in pH was observed when arginine, leucine, and tyrosine were the sources of nitrogen. The change of the pH to neutrality in cultures of the conidial forms grown in a medium containing casein hydrolysate was the same as that observed in previous experiments. However, when glutamic acid was the source of nitrogen, the rapidly-growing, mycelial forms caused the medium to become neutral or slightly alkaline in reaction, while the conidial forms caused little change. This was the only medium in which the rapidly-growing forms caused such a change. Although the conidial forms N, 4a4 and D1 produced moderate growth on this source of nitrogen, particularly on the agar medium, little change in the reaction of the medium occurred.

TABLE VII

HYDROGEN ION CONCENTRATIONS OF LIQUID AND AGAR CULTURE MEDIA CONTAINING FIVE DIFFERENT NITROGEN SOURCES ON WHICH THE SIX FORMS OF *T. mentagrophytes* HAD BEEN GROWN FOR 14 DAYS

Form	Nitrogen source				
	Casein hydrolysate	Glutamic acid	Arginine	Tyrosine	Leucine
	pH	pH	pH	pH	pH
B	5.0–5.5	7.0–7.5	5.0–5.5	5.0–5.5	5.0–5.5
7a14	5.0–5.5	7.0–7.5	5.0–5.5	5.0–5.5	5.0–5.5
4a15	5.0–5.5	7.0	5.0–5.5	5.0–5.5	5.0–5.5
4a4	6.5–7.0	5.5–6.0	5.0–5.5	5.0–5.5	5.0–5.5
N	6.5–7.0	5.5–6.0	5.0–5.5	5.0–5.5	5.0–5.5
D1	6.5–7.0	5.5–6.0	5.0–5.5	5.0–5.5	5.0–5.5

DISCUSSION

The results of this investigation substantiate the belief of Robbins and McVeigh (3) that the pleomorphic forms of *T. mentagrophytes* are due to mutations rather than to the phenomena of heterocaryosis and nuclear separation as proposed by Wilhelm (5). Although each form varied somewhat on the different media, certain growth characteristics, such as the production of either abundant or scant mycelia and numerous or few conidia, remained constant, and no evidence of any nuclear separation was observed. The stock cultures were maintained apparently unchanged for the duration of this study, a period of about 8 months, by transfers to fresh agar slants at weekly intervals.

The order of magnitude of growth of the six forms studied remained approximately the same on all media. This order was as follows: B > 7a14 > 4a15 > 4a4 = > N > D1. Some variation was found in the order of magnitude of growth of the slow-growing, conidial forms, particularly the normal form (N) and variant 4a4. However, as was pointed out previously, these forms yielded such small amounts of growth on some media that slight experimental errors could be responsible for this variation.

The differences in amount of growth between the mycelial and the conidial forms increased when simple nitrogen sources were substituted for complex ones. This can be most clearly seen when the dry weights given in tables III and VI are considered. For example, when casein hydrolysate was the source of nitrogen in a liquid medium (TABLE III), growth of the mycelial variant B was only 2.85 times that of the conidial form N, whereas when asparagine was the nitrogen source, growth of variant B was nearly 24.5 times that of the normal form. This was also true when casein hydrolysate was replaced by individual amino acids (TABLE VI). For example, with casein hydrolysate as the nitrogen source in an agar medium, growth of B was only 2.86 times that of N, but when leucine replaced casein hydrolysate, growth of variant B was 42 times that of N. These results seem to verify the opinion of Robbins and Ma (1) that mycelial pleomorphic forms of *T. mentagrophytes* have developed more effective enzyme systems, that is, systems which function in transforming ammonium salts, asparagine and single amino acids into those amino acids used in the building of protoplasmic proteins.

The amino acids used in this study had been reported by Robbins and Ma (1) to be beneficial to the normal form of this fungus, after 11 days of incubation in the following order: tyrosine > glutamic acid > leucine = phenylalanine > arginine. Since these workers had found that growth on no one amino acid was equal to that on casein hydrolysate when used on an equal weight basis in amounts not exceeding 120 mg. per liter, it seemed desirable to determine whether growth on any one amino acid would be comparable to that on casein hydrolysate if used in quantities containing equivalent amounts of nitrogen. The amino acids were used in considerably larger quantities than had been supplied by Robbins and Ma (1). The results obtained, however, indicated that none of these amino acids when used on the basis of equivalent nitrogen content could support growth to the same extent as casein hydrolysate. This suggests that *T. mentagrophytes* incorporates the amino acids of casein hydrolysate into proteins as units, whereas individual amino acids must be broken down and rebuilt into other essential amino acids before proteins can be synthesized.

The order of availability of the nitrogen of the amino acids when used in quantities containing equivalent amounts of this element differs somewhat from the order found by Robbins and Ma (1) when equal weights of amino acids were used. On the basis of equivalent nitrogen content, the amino acids were beneficial to each of the mycelial forms (B, 7a14 and 4a15) in the following order: glutamic acid > arginine = > tyrosine > leucine. The order was essentially the same for the conidial forms especially in agar media, but some small variation was found when these forms were grown in liquid media. Because of the small size of these mats the variations may have been due to errors in determinations of weights. Phenylalanine, when supplied in the relatively high concentrations used in this investigation, appeared to be toxic to all forms except variant B. The colony which developed in one tube inoculated with 7a14 was probably a pleomorphism. A difference in growth on the amino acids when supplied in equal quantities and when supplied in quantities containing equivalent amounts of nitrogen would be expected because of the differences in amounts of total nitrogen present. How much of this nitrogen is actually available to the organism has not been determined. It is quite possible that some of the amino acids in the rather high concentrations employed in this investigation, and not balanced by the presence of other amino acid in the medium, had an antagonistic or inhibitory effect on the organism. This may account in part for the difference between the relative amounts of mycelia produced on the various amino acids in this investigation and that reported earlier by Robbins and Ma (1). It would be desirable to determine the optimum concentrations of each of these acids for the growth of the various forms.

The differences between weights of mats obtained from liquid and those from agar media in the presence of the same nitrogen source (TABLE VI) were probably due to variations in the oxygen supply available to the organism. When grown in liquid media part of the mycelium was unavoidably submerged and it is possible that the oxygen available was not sufficient to yield growth equal to that on agar. This was especially true for the slow-growing highly conidial forms which appear to have a greater oxygen requirement than the mycelial forms.

The changes in hydrogen ion concentration found in certain of the media are of interest, largely because of the regular manner in which such changes occurred. In media containing casein hydrolysate, asparagine, and urea the slow-growing, conidial forms caused a change in pH from 5.0–5.5 to near neutrality or to a slightly alkaline condition, while the rapidly-growing, mycelial forms caused little or no change. However, when glutamic acid was the source of nitrogen the mycelial forms caused a change in pH from 5.0–5.5 to 6.5–7.0, while the conidial forms had very little effect on the pH. No explanation can be made of this phenomenon at the present time.

SUMMARY

Of the six forms of *T. mentagrophytes* included in this study, the three mycelial forms (B, 7a14, 4a15) were found to be able to utilize nitrogen from inorganic and simple organic sources better than the conidial forms (4a4, N and D1). All forms grew well when complex organic nitrogen sources, such as neopeptone and casein hydrolysate, were supplied. No single amino acid, used in quantities equivalent in nitrogen content, was found to support growth as well as did casein hydrolysate.

A relationship was found between the type of growth of the different forms and the changes which they caused in the hydrogen ion concentration of the culture media. After 14 days of incubation in media containing either casein hydrolysate, asparagine or

urea, the slow-growing, conidial forms had caused a change in pH from 5.0–5.5 to neutrality or to a slightly alkaline condition, while the rapidly-growing, mycelial forms had produced little or no change. However, in the medium containing glutamic acid the reverse was true.

No evidence of nuclear separation was found during the course of this investigation. The results obtained by dry weight determinations indicated that the order of magnitude of growth of the normal form and its variants remained essentially the same on each of the sources of nitrogen, and the cultures of any one form grown on the different agar media exhibited certain constant growth characteristics. These results substantiate the idea that the variants of *T. mentagrophytes* are due to mutations rather than to a heterocaryotic condition.

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A SURVEY OF THE GROWTH REQUIREMENTS OF SOME BASIDIOMYCETES[1]

WILLIAM J. ROBBINS[2]

(WITH 1 FIGURE)

The study of the growth substance deficiencies of microorganisms has proved to be an important field of investigation not only for the biology of the organisms concerned but for the discovery of new vitamins, for bioassay and for determining the steps involved in the synthesis and degradation of important metabolic substances. The present paper reports a preliminary survey of the nutrient requirements of a group of Basidiomycetes, primarily wood destroyers. Most of the cultures used were originally obtained from Ross W. Davidson, Department of Forest Pathology, United States Department of Agriculture, to whom our grateful appreciation is expressed.

Species of a number of the genera included in this survey as well as other Basidiomycetes have been reported to be partially or completely heterotrophic for thiamine and a few have been found to be heterotrophic for both thiamine and biotin. A review of the literature will not be included here but a bibliography for the convenience of interested readers is given at the end of this paper.

In our survey, each of 310 isolations (including 281 species) of Basidiomycetes was grown at 25° C. on an agar medium containing 2 per cent Difco desiccated malt extract and on a basal medium.

The basal medium contained per liter 50 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$ and 2 g. asparagine. To this solution the following trace elements in p.p.m. were added: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo, 0.09 Zn. The agar was purified by extracting Difco agar in a percolater successively with 5 per cent aqueous pyridine, distilled water, 1 per cent hydrochloric acid and distilled water. The agar was then neutralized with calcium hydroxide, washed free of excess calcium, partially dehydrated with 95 per cent ethanol and dried at 40° C.

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[1] The author acknowledges the assistance of Roberta Ma and Ilda McVeigh in the experimental work summarized here. The investigation was supported in part by the Alexander P. Anderson and Lydia Anderson Fellowship Fund of The New York Botanical Garden.

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All of the isolations grew on the malt agar but the growth on the basal medium of 190 of the 310 was extremely poor.

The results of this preliminary survey at the end of one month with the fungi grouped according to genera are given below. The number of species is given in parenthesis.

Genus	Total number species and isolations	Number showing very little growth on basal medium
Armillaria (1)	1	1
Claudopus (1)	1	1
Clitocybe (2)	2	2
Collybia (2)	2	1
Coniophora (2)	2	0
Coprinus (1)	1	0
Corticium (11)	12	9
Cytidia (1)	1	1
Daedalea (6)	7	4
Echinodontium (1)	1	0
Exidia (1)	1	0
Favolus (1)	1	1
Fistulina (1)	1	1
Flammula (1)	1	1
Fomes (43)	45	20
Ganoderma (1)	1	1
Hirneola (1)	1	1
Hydnum (9)	11	10
Hymenochaete (5)	7	4
Hypholoma (1)	1	1
Irpex (2)	2	1
Lentinus (3)	3	1
Lenzites (4)	5	4
Lepiota (1)	1	0
Lycoperdon (2)	2	0
Merulius (3)	3	2
Omphalia (1)	1	0
Panus (4)	5	3
Peniophora (7)	7	6
Phallus (*Ithyphallus*) (1)	1	1
Phlebia (1)	1	1
Pholiota (6)	7	5
Pleurotus (5)	5	0
Polyporus (83)	91	62
Poria (29)	32	17
Ptychogasterm (2)	3	0
Radulum (1)	1	1
Schizophyllu (1)	1	1
Sparassis (1)	1	1
Stereum (21)	27	20
Trametes (11)	11	6

In addition to the 190 mentioned above, a substantial number was estimated to have on the basal medium from one-fifth to one-quarter the amount of growth observed on 2 per cent malt agar.

We had expected that some of the species would grow poorly on the basal medium but we had not anticipated that nearly three-quarters of the 281 species surveyed would find it so unsatisfactory. Many fungi grow very well on this medium.

To define somewhat more definitely the cause of the poor growth on the basal medium, two genera were selected for further investigation: the 62 isolations (56 species) of *Polyporus* which grew very poorly on the basal medium, and the 20 isolations (17 species) of *Fomes*. Each of the isolations was grown on five media prepared with purified agar as follows: 2 per cent malt extract, the basal medium, the basal medium supplemented with a vitamin mixture,[8] with 625 mg. of vitamin-free casein hydrolysate per l. and with both the vitamin mixture and the casein hydrolysate. Observations on the relative growth of each fungus on the various media were made at intervals and final observations were made after one month.

Of the 56 species of *Polyporus,* 40 responded markedly to the addition of vitamins to the basal medium; the growth of 13 of the 17 species of *Fomes* was markedly improved by the addition of vitamins to the basal medium (FIG. 1, A). It appeared, therefore, that nearly three-quarters of the 73 species of *Polyporus* and *Fomes* investigated grew poorly on the basal medium chiefly because of heterotrophism for one or more of the vitamins included in our vitamin mixture. Conversely, the superiority of malt extract agar for these species was primarily because of the vitamin content of the malt. However, although growth on the basal medium supplemented with vitamins was quite satisfactory, few of the fungi developed as rapidly on the synthetic medium as they did on 2 per cent malt extract agar. On the other hand, the growth of some species after one month on the synthetic medium exceeded that on 2 per cent malt extract. Vitamins improved the growth of some of the remaining 20 species but the improvement

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[8] The vitamin mixture contained thiamine, riboflavin, pyridoxine, niacin, para-aminobenzoic acid, pantothenic acid, guanine, hypoxanthine, i-inositol, 2-methyl-1, 4 naphthohydroxyquinone, biotin and pimelic acid.

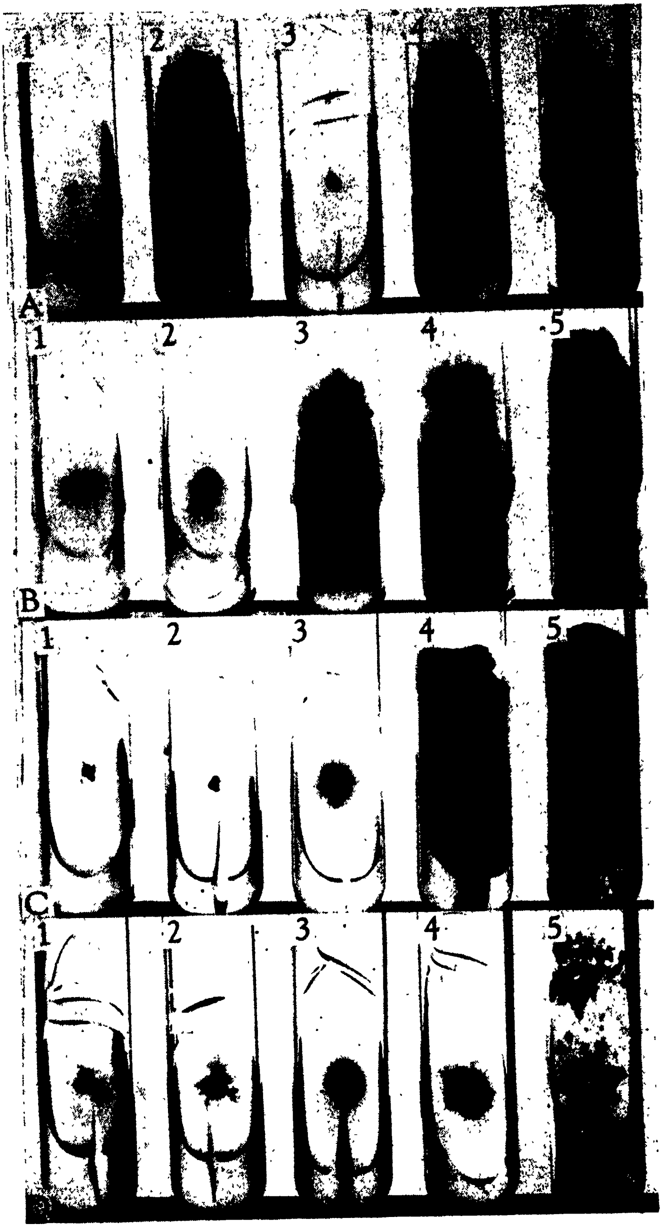

FIG. 1. Fungi grown 35 days on 1, basal medium; 2, plus vitamin mixture; 3, plus casein hydrolysate; 4, plus both vitamin mixture and casein hydrolysate; 5, on 2 per cent malt extract. A, *Polyporus distortus* No. 57059-S; B, *Fomes pomaceus* No. 2283; C, *Polyporus squamosus* No. 39296; D, *Polyporus immitus* No. 71384.

was not great. Their growth on a vitamin-supplemented medium was limited by some other factor.

A few of the 20 species which grew poorly on the medium supplemented with vitamins grew quite well on the basal medium to which casein hydrolysate was added (FIG. 1, B). For these species asparagine appeared to be a poor source of nitrogen and the constituents of casein hydrolysate or products (ammonia, for example) formed therefrom were more satisfactory. Some in this group grew quite satisfactorily when NH_4NO_3 was substituted for casein hydrolysate and asparagine; for others NH_4NO_3 did not replace casein hydrolysate.

There were also some species which did poorly on the medium supplemented with vitamins and on that to which casein hydrolysate was added but grew quite well when both vitamins and casein hydrolysate were included (FIG. 1, C). These species not only found asparagine unsatisfactory for growth but suffered from vitamin deficiencies as well.

A few grew quite poorly on all the media except the 2 per cent malt extract (FIG. 1, D). The superiority of 2 per cent malt extract as compared to our basal medium supplemented with vitamins and casein hydrolysate for these species might be ascribed to the lower osmotic pressure of the malt extract medium,[4] to the presence in the basal medium of injurious material or to the presence in malt of unidentified essential metabolites not included in our vitamin mixture or in casein hydrolysate.

Further investigation is desirable to define the vitamin requirements of these fungi, to determine the reason for the beneficial effect of casein hydrolysate, and to learn why malt extract was in some instances so superior to our basal medium supplemented with vitamins and casein hydrolysate. Judging from the results obtained with *Polyporus* and *Fomes*, a study of nutrient requirements might well be extended to other genera of the Basidiomycetes which grow well on a natural medium but poorly on a basal synthetic medium and to those which have not been successfully cultivated on laboratory media (5).

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[4] The osmotic pressure of 2 per cent malt extract would probably be less than ⅙ that of our basal medium which contained 5 per cent dextrose.

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STEMPHYLIUM LEAF SPOT OF CHINA ASTER

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(with 2 figures)

Commercial cut-flower plantings of China aster [*Callistephus chinensis* (L.) Nees.] near Redondo Beach, California, were found (4) in August, 1946, to sustain serious localized losses from a previously unreported *Stemphylium* leaf spot. The most severe losses occurred in low pockets of fields irrigated by frequent overhead sprinkling; the areas were shielded from the wind by surrounding low hills. There was a progressive increase in the prevalence and severity of the disease downward from the tops of the slopes. In other fields the damage occurred in areas protected by wind-breaks. In such sheltered places the plants remained moist for long periods following irrigation, and infection was thus favored.

A high inoculum potential of *Fusarium oxysporum* f. *callistephi* (Beach) Snyd. et Hans. has been developed in the more desirable level land by repeated aster plantings, making it necessary to grow the locally selected wilt-susceptible types on virgin hill slopes. This in turn has necessitated sprinkling rather than ditch irrigation, and has provided the conditions favorable for the leaf spot.

This paper presents details of the life history of *Stemphylium callistephi* and of the disease it causes.

SYMPTOMS

The fungus causes a conspicuous spotting of leaves, bracts, and sometimes petals and stems. Young leaf spots are brown and nearly circular. As they enlarge, the area surrounding the original brown spot becomes lighter in color, although the advancing margin remains dark; this gives large spots a concentrically zonate appearance (fig. 1, A, B). The lesions are 1–10 mm. in diameter, and they may coalesce to form irregular areas which sometimes become so large as to kill the leaf. Under moist field conditions the centers of the spots have dark spore masses, particularly on the undersurfaces. The dead centers of leaf spots may crack and drop out. Severely affected plants may have so many dead leaves as to obscure the fact that a leaf-spot disease is involved.

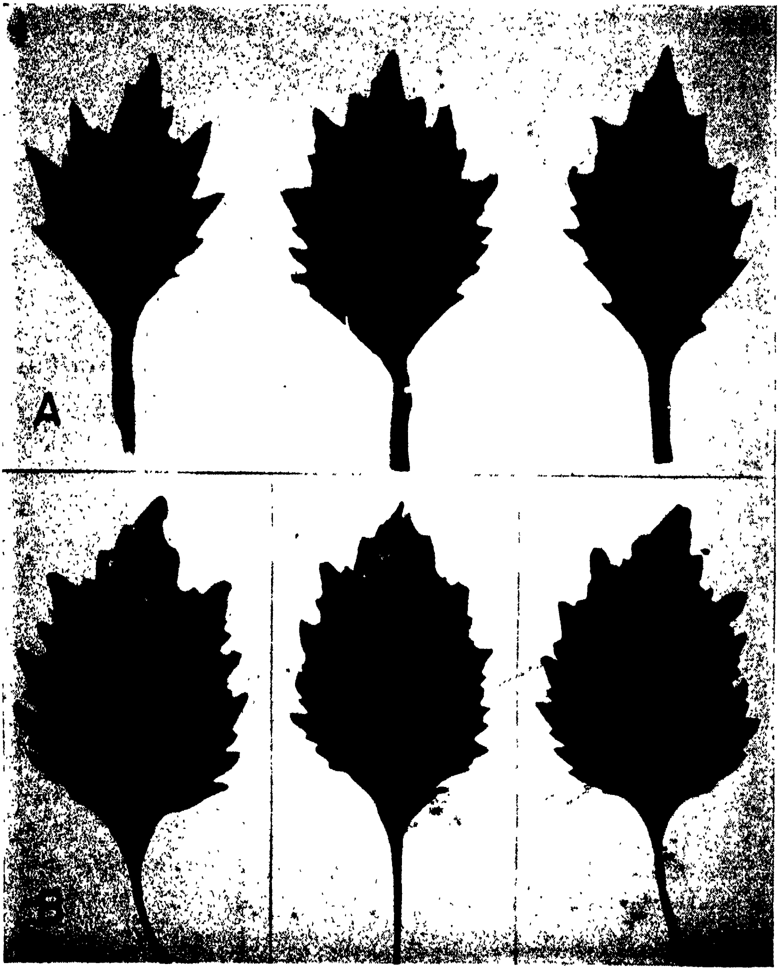

FIG. 1. *Stemphylium* leaf spot of China aster. A, Naturally infected leaves from commercial flower fields. B, Leaves inoculated by atomizing with spores in greenhouse; photographed 15 days after inoculation.

Bracts of the flower head often have many spots similar to those on leaves, and petals may develop brown areas that eventually cause shrivelling. The lesions on flower parts and on leaves render the cut flowers unsalable and specimens without visible infections commonly decay under moist shipping conditions. Shippers, therefore, often refuse to buy asters from a planting known to be infected.

There are several foliage diseases of China aster in this country from which this leaf spot should be distinguished. A leaf spot caused by *Septoria callistephi* Gloyer (7), once important in New York, seems to have become rare in the last two decades, and one caused by *Ascochyta asteris* Gloyer (5) is also now uncommon. The spots caused by these fungi have small dark pycnidia which distinguish them from lesions caused by *Stemphylium*. The leaf blight caused by *Rhizoctonia solani* Kühn is largely restricted to the basal rosette, and coarse strands of mycelium are seen on the surface of decayed leaves (10). Leaf rust [*Coleosporium solidaginis* (Schw.) Thüm.] is easily distinguished by the golden sori on the undersurfaces of the leaves, but the pustules may be killed and enlarged by secondary fungi that produce a distinct spot. A downy mildew (*Basidiophora entospora* R. & C.), reported in Texas (1), can be recognized by downy sporulation on the leaf undersurfaces. Foliar nematode (*Aphelenchoides fragariae* R. Bos) in aster leaves occasionally produces brown lesions sharply limited by the veins. The spotted-wilt virus commonly produces irregular tan blotches in the leaves, and these may show concentric ring patterns; involucral bracts and young apical leaves often show a mosaic-like mottling that differentiates the trouble from *Stemphylium* leaf spot.

THE PATHOGEN

The fungus constantly associated with the disease was an apparently undescribed species of *Stemphylium* of the Eustemphylium type. Conidia are formed on the host only under moist conditions. The conidiophores are brown, septate, 37–134 × 6–11 (average 71 × 8) μ, borne singly or rarely in fascicles of 2 to 3; in cultures and under extremely moist conditions, they may reach a length of 175 μ. The bases are slightly swollen and the distal ends are

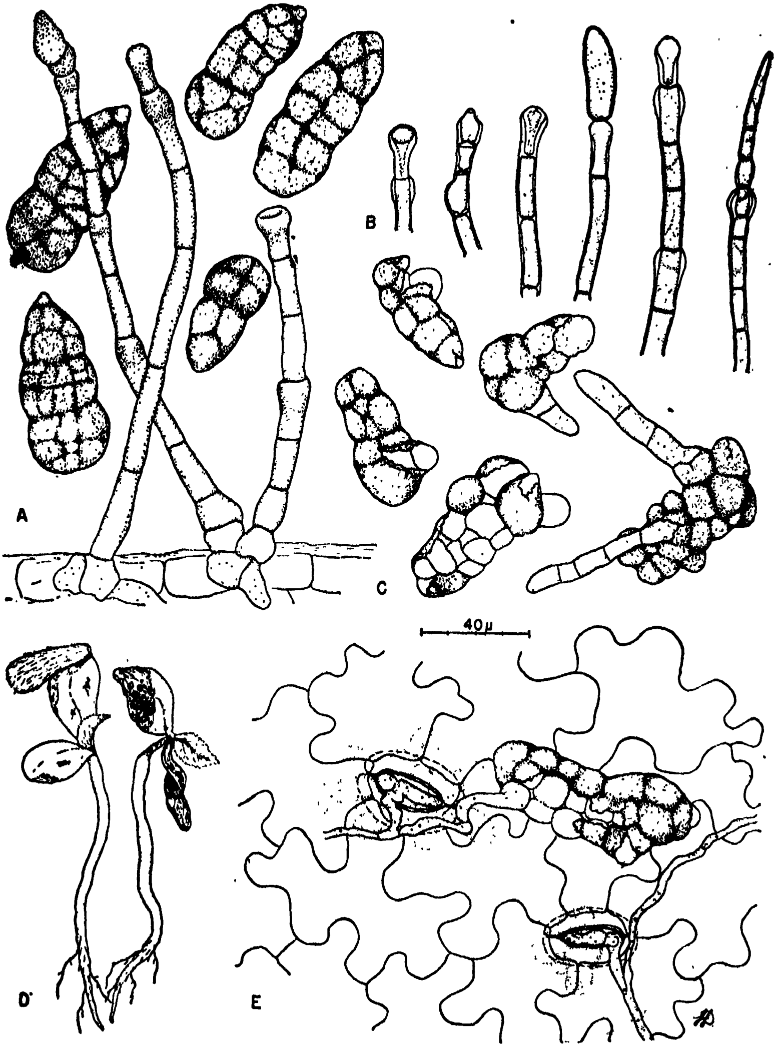

Fig. 2. Camera lucida drawings of *Stemphylium callistephi* n. sp. from leaves of China aster. A, Conidiophores and conidia. B, Detail of swollen tip of conidiophore. Mycelial fragment at right continuing growth. C, Conidia in various stages of germination. D, Aster seedlings with infections of cotyledons from infested seed. E, Infection of aster leaf showing formation of sub-stomatal enlargements. Epidermis stripped off and stained with lactophenol cotton blue.

bulbous, with a diameter of 10–17 (average 13) μ. After a mature conidium is shed, growth frequently continues through the apex of the conidiophore, causing a series of successive swellings that appear darker because of the double wall (FIG. 2, A, B). Branching of the conidiophore was not observed on the host and is not common in culture; when it does occur, it is near the base. The brown muriform conidia are borne singly, have relatively smooth walls, are oblong to obclavate and have a subacute to conical distal end. There are 3 to 10 (commonly 5 to 6) cross septa; the two major ones usually deeply constricted (FIG. 2, A). The longitudinal septa vary in number with the age of the spore. The conidia usually fall within the range of 26–67 × 15–30 (average 47 × 23) μ but may attain a length of 72 μ under moist conditions; in culture they are generally longer and narrower than on the host.

The fungus grows well on potato-dextrose agar and at room temperature a single-spore transplant reaches a diameter of 3 cm. in 5 days. At first a mat of hyaline wavy mycelium is formed; later, dark-green aerial hyphae and masses of dark spores develop in the center of the colony. The underside of the culture is nearly black. When cultures are kept for several months, the surface growth becomes cottony and gray, with white sectors of sterile mycelium. Sporulation, however, is typical and abundant when the cultures are placed outdoors on a north window ledge.

Although mycelial knots are common in old cultures, no ascigerous stage was observed in colonies on potato-dextrose agar or water agar plus cold-sterilized ground aster stems, either in the laboratory or under fluctuating outdoor temperatures.

The fungus was reported previously by us (4) to be *S. botryosum* Wallr. as defined by Neergaard (8) and Wiltshire (12). After examination of our specimens, Dr. S. P. Wiltshire[1] concluded that it was not that species because: (a) no ascigerous stage was produced, whereas *Pleospora herbarum* (Pers.) Rabenh. is commonly formed by *S. botryosum;* (b) conidial walls were smoother than those of *S. botryosum;* (c) numerous spores had two major, rather than one median, cross walls; (d) many spores had a bluntly tapering apex not seen in the rectangular to roundishsquare conidia of *S. botryosum;* (e) spores were more elongated and larger than those of *S. botryosum.* Dr. P. Neergaard[2] independently confirmed this conclusion. It was pointed out by Dr. Wiltshire[1] that the spores of this fungus resembled those of *Macrosporium nigricantium* Atk. from cotton (2, p. 62; 3, p. 17; fig. 4 of pl. 1) in the pointed apices and relatively smooth walls; they differed in having two, rather than one, major cross walls, and in the larger spore size. Following comparative studies of our fungus with several exsiccati of *M. nigricantium,* he suggested that the aster pathogen be described as a new species.

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[1] Letters of November 25, 1947 and February 22, 1949.

Young plants of an American Upland cotton (*Gossypium hirsutum* L.)[3] remained healthy following inoculation with the fungus during glasshouse tests in which asters became infected. No cultures of *M. nigricantium* were available for reciprocal inoculations.

Because of these various lines of evidence the fungus is regarded as new, and is here described.

Stemphylium callistephi sp. nov.

Maculis brunneis, circinato-striatis, subcircularibus, 1–10 mm. in diam., postea in areas magnas coalescentibus, fructificatione praecipue hypophylla; conidiophoris fuscis, septatis, non ramosis, in substratum singulatim aut raro in fasciculos 2–3 congestis, 37–134 × 6–11 μ, inaequalibus, basibus crassiusculis, interdum articulatis fuscis, apice bullato; conidiis acrogenis, solitariis, brunneis, oblongis ad obclavatis, basi rotunda aut subtruncata, apice subacuto aut conico, plerumque 3–10 (medio 5–6) septato-muriformibus, ad 2 septa majora constrictis, septis longitudinalibus in numero variabilibus, 27–67 × 15–30 μ.

Habit in living leaves, bracts, and flowers of *Callistephus chinensis* Nees. near Redondo Beach, California, August 16, 1946.

Specimens of *Stemphylium callistephi* have been deposited in the following herbaria: Department of Botany, University of California, Berkeley; Division of Plant Pathology, University of California, Los Angeles; Department of Plant Pathology, Cornell University, Ithaca, New York; Farlow Herbarium, Harvard University, Cambridge, Massachusetts; Division of Mycology and Disease Survey, Plant Industry Station, Beltsville, Maryland; Commonwealth Mycological Institute, Kew, England; Phytopathological Laboratory, J. E. Olsens Enke, Copenhagen, Denmark.

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[2] Letter of December 4, 1947.

[3] Seed was kindly supplied by Dr. G. M. Armstrong.

There have been few other reports of *Stemphylium, Alternaria,* and *Macrosporium* on China aster. *Stemphylium botryosum* was reported on seed in Denmark (8), but pathogenicity on this host was not demonstrated. *Pleospora herbarum* and an *Alternaria* sp. were reported (5) to cause seedling blight of China aster in New York under excessively wet conditions. *Alternaria zinniae* Pape and *A. tenuis* auct. sensu str. also have been reported on this host by Neergaard (8). *Macrosporium florigenum* Ell. & Dear. was collected on China aster flower heads in London, Canada, in September, 1891 (J. Dearness No. 3097) and August, 1892. *M. caudatum* C. et E. also was collected in flower heads of China aster at the same place in October, 1891 (J. Dearness No. 816b). We have examined specimens of these Canadian collections and found the fungus to be a long-beaked Alternaria. Apparently, there is no previous record of a *Stemphylium* pathogenic to this host.

LIFE HISTORY

Single-spore cultures were made from dilution plates of spores produced on the leaf spots and pathogenicity was demonstrated by atomizing healthy China aster plants in the glasshouse with a suspension of spores from pure cultures. The plants were held under high humidity for 2 days. Typical leaf spots began to appear 5 days after inoculation (FIG. 1, B).

Parasitic relations with the host were not established unless the atomized plants were kept moist for 48 hours; this unusually extended infection period was investigated. Conidia, germinated on water agar and on aster leaves floated on 2 per cent sucrose in petri dishes, were examined after various intervals. During the first 24 hours the spores swelled conspicuously and irregularly split the outer wall (FIG. 2, C, E). Germ tubes appeared after 27 hours at laboratory temperatures and grew over the surface of the leaf. Spores from extremely moist cultures were swollen and irregular; they germinated in only 2 hours without the swelling process exhibited by spores grown under dry conditions. After 52 hours, 5 of 18 germ tubes of spores examined in one series had entered stomata and formed characteristic, swollen, branched, sub-stomatal structures (FIG. 2, E). As with *S. botryosum* on sainfoin (6), no direct cuticular penetration by germ tubes from spores was observed. It may occur under some conditions, however, as has been reported for *S. botryosum* on alfalfa and red clover (9) and *S. solani* Weber on tomato (11). Penetration between epidermal cells by germinating bits of mycelium was observed after 24 hours. Injuries are not necessary for infection. After the surrounding host cells have been killed, the sub-stomatal enlargements give rise to mycelia which penetrate intercellularly through tissue, killing the cells as they advance.

The prolonged incubation period of this fungus explains the limitation of infection in the field to low areas that remain moist for long periods because of protection from drying wind.

Seed Transmission. Because of the copious sporulation on flower heads conidia probably adhere to the seed. It is also probable that the fungus grows from a petal spot through the tissue and penetrates the attached seed, producing internal infection.

External transmission and seedling infection was demonstrated experimentally. Aster seed was surface sterilized with sodium hypochlorite, dried, dipped in a suspension of *Stemphylium* spores, and again dried; numerous conidia were caught in the bristles of the seed, even in those truncated by commercial cleaning operations. Such infested seed was planted in sterile black peat where the conidia germinated and mycelium grew on the coats, sporulating copiously by the time the seedlings emerged. Because of the epigeal germination of aster seed, the conidia produced on the coat can be carried by air currents and spattering water to the hypocotyl and to adjacent seedlings. When the coat remains on the cotyledons (FIG. 2, D), direct infection by mycelial growth may occur. Infections develop on the hypocotyl as chestnut brown streaks and pin-point lesions. The cotyledons are frequently infected and either develop numerous spots or decay and kill the growing point (FIG. 2, D); conspicuous sporulation then occurs.

The cut-flower growers in the Redondo Beach area produce a number of locally developed varieties and save the seed from plants selected in the fields, a situation that undoubtedly accounts for perpetuation of the pathogen. Carryover in soil or refuse is less important because rotation is practiced to reduce loss from *Fusarium* wilt.

Commercial aster seed fields in California were carefully examined for this disease but it was not found. As these fields are ditch irrigated, such freedom from the pathogen would be expected. It is possible that with overhead sprinkling or a wet summer, the fungus would infest and be disseminated with commercial seed. Under the rainy conditions and cloth-house florist culture practiced in the eastern states, the disease might become important if introduced there.

CONTROL

Because the disease is restricted to areas having little air movement, control under the semi-arid southern California conditions is relatively simple. Planting in low protected areas is to be avoided, and ditch rather than overhead irrigation should be practiced. If the disease becomes established in more humid areas, further measures such as seed treatment or fungicidal sprays might become necessary.

SUMMARY

Stemphylium callistephi n. sp. is described. It caused a previously unreported leaf spot of China aster in 1946 in cut-flower fields of southern California.[4]

For the fungus to sporulate on the host, a prolonged period of high humidity is required. The foliage must be wet for 48 hours for infection to occur. This prolonged incubation period restricts the disease to low sheltered areas in fields where overhead irrigation is practiced. Control is possible by avoiding such areas and by practicing ditch irrigation.

Seed transmission of the pathogen was experimentally demonstrated.

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[4] The disease was found in several commercial aster fields in San Mateo and Santa Clara Counties of central California in March, 1950. It occurred in open fields and cloth houses, particularly the latter, under conditions of excessively heavy irrigation.

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THE GENUS TILLETIOPSIS[1]

GEORGE NYLAND[2]

(WITH 5 FIGURES)

In making isolations from leaves of various plants using the spore-fall method, many cultures of a fungus identified as *Tilletiopsis* Derx were obtained along with those of species of *Sporobolomyces* (3). Macroscopically the two genera can be distinguished by the white or cream color of the colonies of *Tilletiopsis* in contrast to the salmon or pink color of the colonies of *Sporobolomyces.* In figure 1 is shown the appearance of the colonies on malt agar plates formed as a result of ballistospores being forcibly discharged from the surface of leaves affixed to the inside of petri dish covers. In most cases, the colonies of *Sporobolomyces* were in the majority, but those of *Tilletiopsis* were usually present in considerable numbers.

At least two species of *Tilletiopsis* were isolated in this manner from leaves of 28 different hosts, including vegetables, common weeds, and several species of deciduous trees, during the summer of 1947. The same two species were isolated again from a dead leaf of *Acer macrophyllum* Pursh collected in February, 1948, near Kent, Washington, by G. W. Fischer.

Derx (1), who first described fungi of this type in 1930, provisionally suggested the name *Tilletiopsis* because of their resemblance, morphologically, to certain species of *Tilletia.* He designated, by numbers, five strains or isolates which, according to him, were readily distinguishable on the basis of spore size. The spore sizes of his five strains were, respectively, $9 \times 2\,\mu$; $7 \times 1.6\,\mu$; $15 \times 2\,\mu$; $13.4 \times 2.3\,\mu$; and $12 \times 3\,\mu$. The two species isolated by the writer have spores averaging $9 \times 1.8\,\mu$ and $14 \times 2.4\,\mu$. There is probably no significant difference in spore size between the writer's small-spored species and Derx's strain 1. Neither does there seem to be significant difference between the writer's large-spored species and Derx's strains 3 and 4.

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[1] Published as Scientific Paper No. 888, Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman.

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Recently Derx (2) validated the genus *Tilletiopsis,* giving a brief Latin description, and designating his strain 4 as the type species for the genus. This strain is no longer available at the "Centraalbureau," so further comparison with the writer's large-spored species was not possible. Since only spore size distinguishes Derx's strains, and since his strain 4 is comparable to the writer's large-spored species, they are considered to be the same. The name *Tilletiopsis washingtonensis* is proposed and considered to be the type species for the genus. For the writer's small-spored species, the name *Tilletiopsis minor* is proposed.

For convenience, Derx's genus description (2) is repeated here:

Tilletiopsis Derx.

Mycelium hyalinum, septatum, repens, sterigmata in aera ascendentia formans. Sporae solitariae, falcatae, leves, hyalinae.

Species typica: Tilletiopsis spec. No. 4 Derx.

Tilletiopsis washingtonensis sp. nov.

Ballistosporae quae denuo gemmandeque repetitione generant, flexae, falci similes, hyalinae 8–19 × 2–2.6 μ, circa 14 × 2.4 μ, non mediae in sterigmatibus aut e ballistosporis aut e mycelio ascendentia generatae. Cellae filiformatae gemmatae, 19–34 × 2–3 μ; quae similis fermento cultura consistunt. Cellae myceliales inflatae et chlamydosporae in veteris culturae, praesertim si substratum siccavit. Chlamydosporae hyalinae, 12–15 μ linea media, plerumque globosae, sive tempestivae, sive solae sive in serie. Culturae in hordeo-gelidio corneo sive candidus sive subflavus colore, ut colore subflavus aut luridus manet.

Hab.: Superficies foliorum herbarum multarum, vivarum aut mortuarum, vicinia Puyallupi, Washingtoni, aestate, 1947. **Typus** est A. S. 20240.[3]

Ballistospores reproducing by budding and by repetition, curved, sickle-shaped, hyaline 8–19 × 2–2.6 μ, average 14 × 2.4 μ, produced eccentrically on sterigmata which arise either from other ballistospores or from the mycelium. Budded cells filiform, 19–34 × 2–3 μ; forming a yeast-like colony. Inflated mycelial cells and chlamydospores present in old cultures, especially if dehydration of substratum has occurred. Chlamydospores hyaline, 12–15 μ in diameter, usually globose when mature, solitary or in chains. Colony on malt agar white to cream in color, soft, cheesy consistency when young, becoming firmer with age, color remaining ivory to light tan.

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[3] Type material deposited in the Mycological Herbarium, Plant Pathology Department, State College of Washington, Pullman, Washington. A. S. numbers refer to the accession series of that herbarium.

Habitat: Surface of leaves of many hosts, living or dead, in vicinity of Puyallup, Washington, summer, 1947.

Tilletiopsis minor sp. nov.

Ballistosporae denuo gemmandeque repetitione generant, hyalinae, flexae, 5.8–14 × 1.5–2 μ, circa 9 × 1.8 μ. Cellae filiformatae gemmatae, directae, plerumque aliquantum ballistosporis. Ballistosporae non mediae ad sterig-

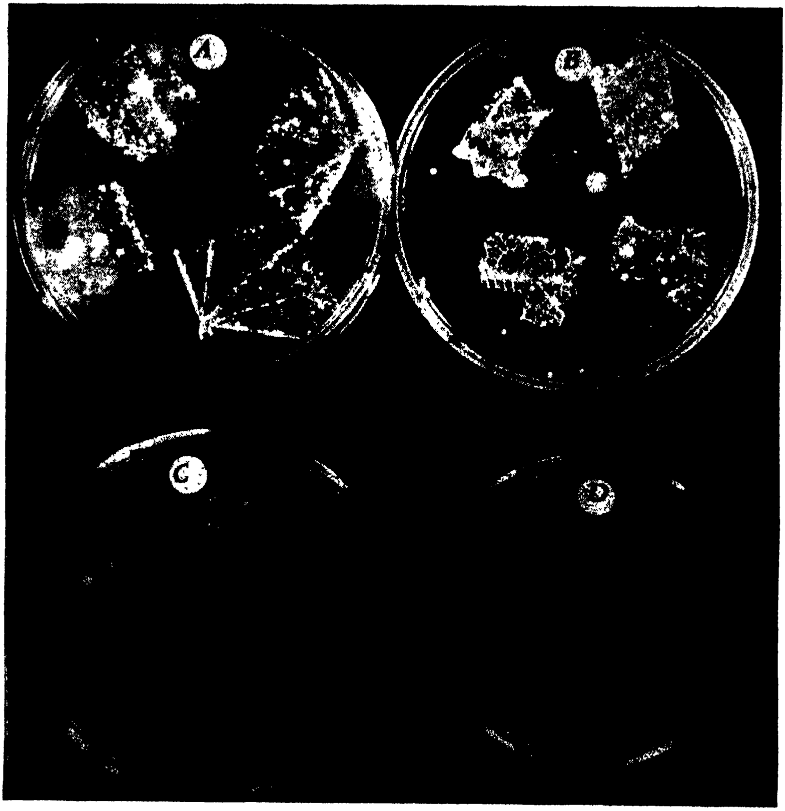

FIG. 1. *Tilletiopsis* spp. and *Sporobolomyces* sp. A. Pattern of colonies originating from fragments of living leaves of *Phaseolus vulgaris* attached to inside cover of malt agar plate. B. Colonies originating from Hubbard Squash leaves. C. From Sweet Corn. D. From Washington variety of Red Raspberry.

mata figetae, sterigmata sive e mycelio sive e ballistosporis aliis sive e cellis gemmantis ascendunt. Mycelium plerumque molle, septatum, hyalinum sive novellum sed fusciore sive tempestivum, columae grandiores lentae et chlamydosporae formatae in mycelio, terminal aut intercalary, hyalinae 7–15 μ linea media, sive globosae sive clavatae aut obovatae, sive solae sive in serie.

Hab.: Superficies foliorum herbarum multarum, vivarum aut mortuarum, vicinia Puyallupi, Washingtoni, aestate, 1947. **Typus** est A. S. 19961.[3]

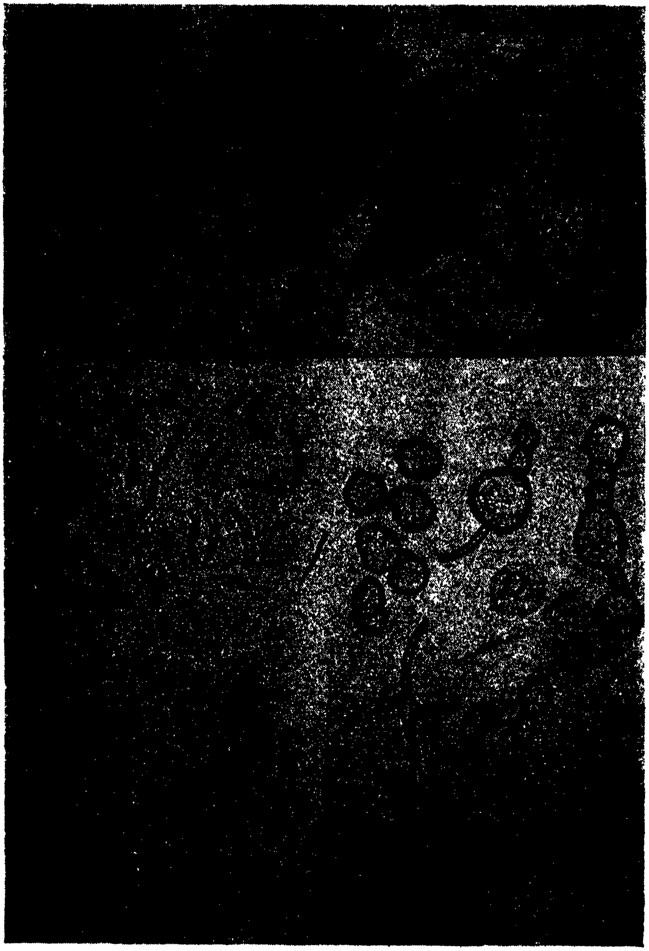

FIG. 2. *Tilletiopsis washingtonensis*. A. Photomicrograph of ballistospores and budded cells showing uninucleate condition. B. Camera lucida drawing of ballistospores. C. Budded cells, and D. chlamydospores showing variation in size. A, approx. × 800. B, C, D, approx. × 500.

Ballistospores reproduce by budding and by repetition, hyaline, curved, 5.8–14 × 1.5–2 μ, average 9 × 1.8 μ. Budded cells filiform, straight, usually somewhat larger than the ballistospores. Ballistospores attached to sterigmata eccentrically, sterigmata arise either from the mycelium or from other ballistospores or budded cells. Mycelium mostly delicate, septate, hyaline when young but becoming darker with age, older colonies tough and cartilaginous. Chlamydospores formed on mycelium, terminal or intercalary, hyaline 7–15 μ diameter, varying from globose to clavate or obovate, produced singly or in chains.

Habitat: Surface of leaves of many hosts, living and dead, in vicinity of Puyallup, Washington, summer, 1947.

This species differs from *T. washingtonensis* by its smaller ballistospores, darker colony color, and the cartilaginous consistency of the colony.

The nuclear condition of the ballistospores of *T. washingtonensis* and *T. minor* was investigated. The ballistospores and vegetative budded cells proved to be uninucleate (FIG. 2, A). The chlamydospores (FIG. 2, D; 4, B) proved difficult to stain, but in those observed there appeared to be a single nucleus. These were very likely fully matured spores, since they were obtained from an old, dehydrated slant culture. Whether or not a binucleate stage occurs in young chlamydospores has not been determined.

The general description given by Derx (1) of these fungi has been essentially confirmed. He reported them as having a delicate, branched, mycelium which eventually formed felted, coherent, cartilaginous masses at first white or tan in color, later becoming light to dark brown, the intensity varying among the different isolates. The fine mycelium gave rise to sterigmata on which were borne pitted, curved, sickle-shaped ballistospores which were forcibly discharged at maturity in the same manner as those of *Sporobolomyces.*

Derx does not mention having observed the ballistospores of his isolates of *Tilletiopsis* budding. In both *T. washingtonensis* and *T. minor* the ballistospores of fresh isolates bud profusely, forming yeast-like colonies with little or no mycelium present. As the colonies become older, more and more mycelium is evident. Transfers from young newly isolated colonies yield only the yeasty, budding type (FIG. 3, A). However, transfers from older colonies yield, in addition to the budding type, a mycelial type in which the ballistospores are produced on short sterigmata from the mycelium (FIG. 3, B). The budded cells are somewhat longer than the

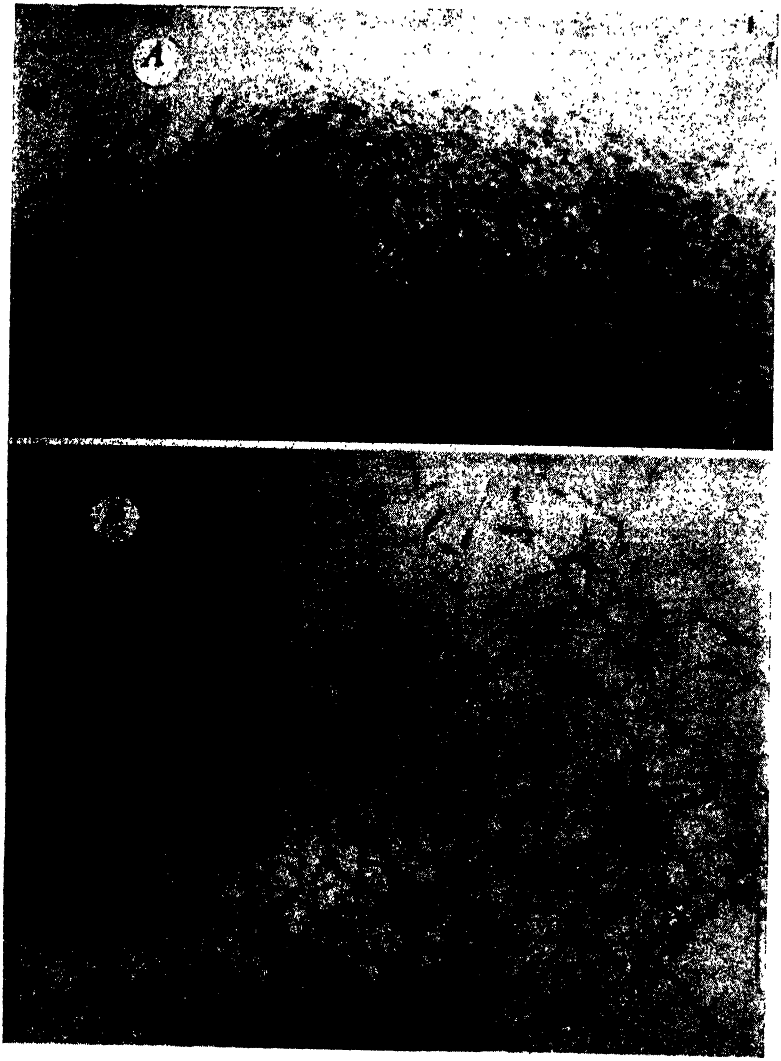

FIG. 3. *Tilletiopsis minor.* A. Mucous or budding type colony. Approx. × 300. B. Mycelial type colony. Approx. × 300. Ballistospores can be seen lying on the agar and also attached to mycelium. Both colonies originated as single spores from the same monosporic culture.

ballistospores and are more or less straight, rather than falcate (FIG. 2, B, C). It is possible that Derx made his observations

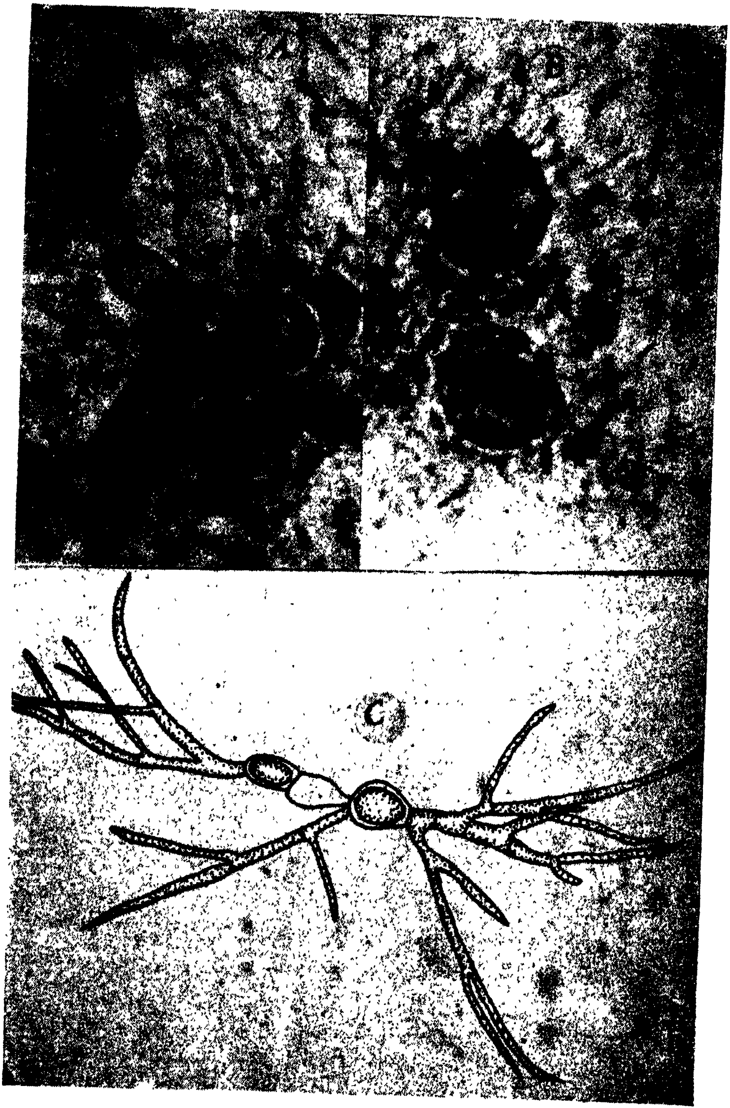

FIG. 4. *Tilletiopsis washingtonensis.* A. Swollen hyphal cells in early stages of chlamydospore formation. B. Mature chlamydospores. Approx. × 1000. C. Chlamydospores germinating on water agar. Approx. × 600.

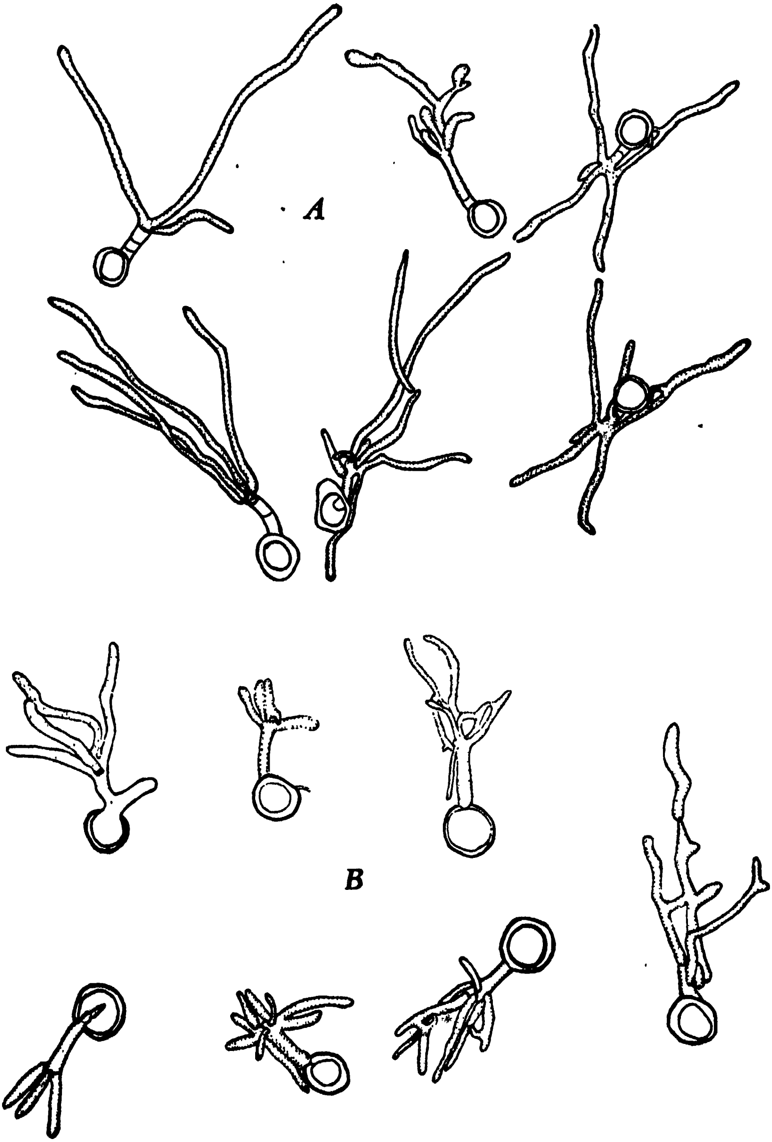

Fig. 5. *Entyloma compositarum.* A. Germinating chlamydospores from *Brauneria purpurea* coll. near Janesville, Wisc., C. G. Shaw and H. C. Greene 7/23/47. B. Germinating chlamydospores from *Heiracium cynoglossoides* var. *medicaule* coll. Mt. Shasta, W. B. Cooke 7/10/47.

using old cultures and therefore failed to observe the budding phase characteristic of freshly isolated colonies.

Derx also makes no mention of chlamydospores formed on the mycelium (FIG. 4). Chlamydospores were observed in old, somewhat dehydrated cultures of both *T. washingtonensis* and *T. minor*. They were usually found in greater abundance in cultures of *T. washingtonensis* than in those of *T. minor*. The chlamydospores bear a striking resemblance to those found in cultures of *Entyloma compositarum*, both as to size and general appearance. The chlamydospores of *T. washingtonensis* and those of *E. compositarum* were compared as to their method of germination. Considerable difference was readily apparent, but there were also certain similarities noted.

At room temperature on water agar, chlamydospores of *E. compositarum* germinated by forming a germ tube which gave rise to several branches (FIG. 5). Hyphal fusions between these branches occurred, and when the germinating spores were transferred to malt agar, continued growth resulted in the formation of mycelial colonies. Within a week to ten days, typical ballistospores (conidia) were produced and forcibly discharged. Chlamydospores were formed in the mycelium and were indistinguishable from those formed in the host.

The chlamydospores of *Tilletiopsis washingtonensis* germinated by producing a mycelium consisting of much branched or budded vegetative cells (FIG. 4, C). No hyphal fusions, such as occurred in *E. compositarum*, were noticed. However, as in the case of *E. compositarum*, ballistospores were produced from the mycelium within ten days. Somewhat later, chlamydospores were observed in the mycelium.

Tilletiopsis in culture closely resembles colonies of certain species of *Ustilago, Tilletia,* and *Entyloma*. In fact, there is sufficient morphological similarity between *Tilletiopsis* and *Entyloma* to entertain the thought that they might be identical. However, the fact that *Tilletiopsis* was isolated from the surface of apparently healthy leaves of 28 unrelated hosts, and even from a dead, fallen leaf of one host during mid-winter, is considered good circumstantial evidence that these fungi are not species of *Entyloma*.

Not only were they present on almost every host collected, but they were present in abundance. Nevertheless, there is nothing to preclude the possibility that certain species of *Entyloma* might be able to develop saprobically on the surface of leaves of immune plants. A distinct difference between *Entyloma* and *Tilletiopsis* in culture is that the ballistospores of *Entyloma* do not bud in a yeast-like manner. In the two species of *Tilletiopsis* isolated by the writer, this budding of the ballistospores resulted in the formation of mucous, yeast-like colonies consisting of filiform cells.

Since species of *Tilletiopsis* have not been proved parasitic, they could hardly be classified as Ustilaginales under the present concept of that order. Their resemblance to *Entyloma* suggests the possibility that they may represent non-parasitic forms of the "white" smuts. Taken as a related group, *Sporobolomyces, Sporidiobolus, Itersonilia,* and *Tilletiopsis,* may be remnants of fungi that were the progenitors of the Ustilaginales. Extensive inoculation experiments might prove them parasitic, in addition to their being free-living saprophytes.

Lack of parasitism alone is hardly sufficient reason for excluding *Tilletiopsis* from the Ustilaginales, in the opinion of the writer. However, a great deal more work is necessary with this group to obtain a complete picture of the nuclear history and life cycle before further classification is attempted.

Cytological work with *Tilletiopsis* is currently in progress by V. M. Cutter, Jr., at Yale University.[4]

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[4] Personal communication with Dr. V. M. Cutter, Jr., Osborne Botanical Laboratory, Yale University, New Haven, Connecticut.

A STUDY OF PEZIZA BRONCA PECK*

Bessie B. Kanouse

(with 6 figures)

Peziza bronca Peck is a North American species of the Discomycetes about which but little has been reported. It was first collected by Dr. Peck in New York and was described by him in 1875.

In the summer of 1948 Dr. A. H. Smith and members of his collecting party made twenty-one excellent collections of a Discomycete in Mt. Rainier National Park, Washington, that corresponded in many respects to Peck's description of *P. bronca.* In addition to these collections the writer had six made elsewhere in the United States that were like the fungus from Mt. Rainier. One collection was made by F. B. Cotner in Colorado, two were made in Wyoming by the writer and three were made in Michigan by A. H. Smith. There were important features in these specimens pertaining to the spores and paraphyses that were not mentioned by Peck (**1875**) or by Seaver (**1928**) who placed the species in the genus *Geopyxis.* Through the kindness of Dr. Homer D. House, New York State Museum, Albany, New York, a loan of the type collection of *P. bronca* was obtained. A comparative study was then made, the results of which showed that the heretofore unreported conditions found in the spores and paraphyses of our specimens were found also in the type specimens. Thus was established the fact that all of our collections should be considered *P. bronca.*

An emended description of the species is given to incorporate the new data. Since the morphology of the species allies it closely with other species of *Pustularia,* a transfer to that genus is proposed.

Pustularia bronca (Pk.) Kanouse comb. nov. Figs. 1–6

Apothecia sessile, sometimes arising from a basal mass of compact hyphae soil and debris, single or in groups 2–3, deep cupshaped or, if in groups, flattened irregularly by mutual pressure, 1–3.5 cm. in diameter, 0.5–1.5 cm. in depth, fleshy, more or less fragile on drying, gray, pallid to creamy, occasionally with tints of deep olive buff throughout when fresh, drying clay color outside, cinnamon buff to pale yellow inside, margin crenulate; hypothecium pseudoprosenchymatic, composed of septate hyphae that are branched, densely interwoven, more or less parallel to the hymenium, becoming pseudoparenchymatic toward the outside, forming an excipular layer, excipular cells large, subglobose to hexagonal in shape, thick-walled, the outermost layers giving rise to the aggregations of cells which form the pustules on the outer surface, pustules large and thickly set on the upper surface of the apothecia, less frequent below, hairs not present; 2 sizes of asci present, the large ones cylindric, 275–350 × 14–16 μ; the small ones 250 × 12–14 μ, both sizes 8-spored and giving no blue coloration in iodine solution; spores of two sizes in separate asci, large spores in large asci 20–24 × 12–14 μ, elliptical, smooth, hyaline, with one or more (frequently 2) large oil globules, usually with a few small ones, uniseriate, frequently slightly overlapping in the asci, small spores in small asci 12–14 × 8–9 μ, elliptical, smooth, hyaline, with 2 distinct oil globules; paraphyses various, of two distinct types, one simple, forked below the middle usually but once, with clavate apices, hyaline, septate; the second type showing various types of irregular thickenings and branching including those with simple bends to knobbed irregularities to fine antler-like apical branching, hyaline, septate, usually extending beyond the asci and frequently adhering in bunches.

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* Papers from the University Herbarium of the University of Michigan.

FIG. 1. *Pustularia bronca.* Apothecia ⅔ nat. size.

Material studied: *Peziza bronca* Peck, type collection, coll. C. H. Peck, New York; Michigan, A. H. Smith nos. *6514, 25[illegible]9, 20479*; Colo., col. F. B.

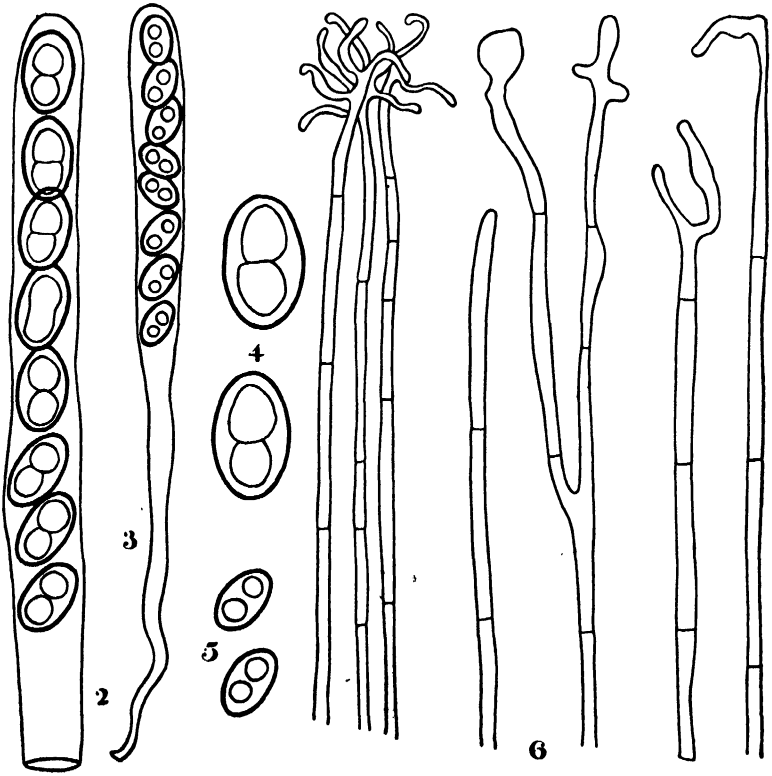
Cotner, 1920; Wyo., B. B. Kanouse, Aug. 27 and Sept. 1923; Mt. Rainier Nat. Park, Wash., A. H. Smith nos. *28922, 29296, 29344, 29880, 29533, 28884, 29614, 29470, 29608, 29285, 34277;* Mt. Rainier National Park, Wash., H. A. Imshaug nos. *764, 757, 825, 1035, 1093, 1230;* E. G. Simmons, same locality, nos. *1689, 1853, 2117, 1849.*

DISCUSSION

The gross morphology of all of the specimens examined was surprisingly uniform. The sessile, deep, cup-like apothecia with warty-rough exterior and crenulate margin; the gray to pallid color (fresh) becoming tinted yellowish inside (dry) make the species distinctive. But it is the microscopic morphological characters that make this species unique. Two unusual features in the structure of the hymenium that were observed were a condition of heterospory and the presence of two types of paraphyses.

The heterosporous condition was found in all of the 29 collections. In many of the asci in the apothecia examined the spores were exactly as Peck reported them; elliptical, smooth, hyaline, 20–24 × 12–13 μ, containing 1 or 2 oil globules. These spores were uniform in size and shape and the asci were 8-spored. There was no evidence that either the spores or the asci were abnormal. In microscopic mounts taken from a single apothecium in which these large spores were seen, there were also other 8-spored asci in which the spores were decidedly smaller. These also appeared to be normal, fully developed spores and from any observable criteria could not be called immature. These spores were elliptical, smooth, hyaline, and measured but 12–14 (rarely 16) × 8–9 μ, and contained 2 distinct oil globules. No asci were observed in which there was a mixture of the two sizes of spores. In all of the apothecia there was a greater proportion of the large spores than of the small ones, yet in many mounts the small spores were present in such numbers as to be definitely conspicuous. As stated above this heterosporous condition was present in the type material. There were fewer small spores in the type than in most apothecia studied. The significant fact is that they were found in the type collection along with the kind of spores that were reported originally for the species.

The condition of heterospory is exceedingly rare in Discomycetes. There are certain genera as, for example, *Thecotheus* and *Rhyparobius,* in which the number of spores is known to be variable and in which the size is thus partly correlated with the number produced in the asci. Also it is a common experience to those familiar with Discomycetes to find asci normally containing 8 spores in which some of the spores disintegrate and the remaining spores become larger than the spores in the other normal 8-spored asci in the same apothecia. But these situations are not comparable to that just described for *P. bronca*. The writer is unaware of a similar condition elsewhere in the Discomycetes. Erichsen (1940) reported a case in a lichen which he named *Buellia heterospora* in which he found asci with eight ellipsoid two-celled spores and other asci in which the 8 spores were nearly globose. That the heterosporous condition was found in the twenty-



FIGS. 2–6. Figs. 2 and 4, ascus and spores representing the large size; figs. 3 and 5, ascus and spores representing the small size; fig. 6, various types of paraphyses.

nine collections studied which represent a distribution in the United States from coast to coast and over a period of 75 years, indicates that the condition must be considered as relatively fixed for the species and that it represents a good diagnostic character.

The second character to be discussed concerns the paraphyses. Peck did not describe them but he did illustrate them. He showed unbranched paraphyses with simple clavate apices. He overlooked the forking and the unusual antler-like apical branching that was found in his type material. Seaver (l.c.) likewise omits mention of this condition.

The irregularities of the apices are quite remarkable. They vary from a simple bend or thickening to a knobbed head having several short stout branches to a branching that is delicate and antler-like. Not all of the paraphyses in one apothecia show this peculiarity. Many of them are simple and clavate; however, the curious branching is very marked in some apothecia and in those in which it is not predominant, the characteristic is indicated by slight bifurcation or slight bends. In the event that branching is profuse, the paraphyses are bunched together due to the interlacing of the branches. Often these clumps extend beyond the asci to a distance of 20–30 μ and form such a covering for the asci that the structure resembles a true epithecium. Boudier (**1905–1910**) illustrated this peculiar branching for *Pustularia ochracea* Boud. In *Otidea abietina* Fuck, there is a characteristic branching of the apices of the paraphyses but it is not like that found in *P. bronca*. The writer (**1944**) reported the finding of two kinds of paraphyses in *Pseudociboria umbrina* Kanouse. The differences here were due to color and form. One kind was hyaline and filamentous, the other kind was dark brown and cylindrical. In *P. bronca* the paraphyses are all hyaline.

The hypothecial and excipular structures are similar to those in other species in the genus *Pustularia*. The hypothecium is composed of intertwining hyphae that, in general, run parallel to the hymenium. These hyphae change to a cellular tissue toward the margin and the outermost layers of this tissue give rise to the aggregations of cells that are the pustules. These warts are larger and more abundant on the upper one-half of the apothecia than on the lower part. There are no hairs or hair-like extensions of the outer cells such as Nannfeldt emphasizes for *P. catinus* and Boudier describes for *P. ochracea.*

The apothecia are sessile. Frequently the mycelium at the base becomes intermixed with soil and debris and forms an irregular mass or ball. These masses are not stipes.

Nannfeldt (1938) discussed the morphology of *Pustularia catinus* from European specimens. He placed *P. ochracea* in synonymy with it partly on the supposition that *P. catinus* may prove to be a collective species including a number of "petites espèces." Be that as it may, *Pustularia bronca* is sufficiently distinct from *P. catinus* so that its status as a species seems well established. *P. catinus* is a stipitate fungus with a hairy excipulum.

P. bronca shows close relationships to species in the genus *Pustularia* Fuckel as emended by Boudier (1907) and the transfer to this genus seems logical. Nannfeldt (l.c.) suggested this possibility but did not make the transfer.

Seaver's treatment of the species is not tenable. His description, based as it was upon a combination of Peck's description and the description of *Aleuria humicola* Boud., which he mistakenly supposed was synonymous with *P. bronca,* does not represent a single species. *A. humicola* is quite a different fungus. It is described and illustrated as being yellow, and as having asci that turn blue in iodine. The apothecia are shown expanded and the exterior of the cup is not warty.

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STUDIES IN THE GENUS CINTRACTIA. I. C. MONTAGNEI AND RELATED SPECIES

LEE LING

The genus *Cintractia,* since its establishment in 1883 by Cornu, has accumulated around one hundred species with rather heterogeneous characters. Many of the species have never been restudied and misinterpretation of certain older ones has been long perpetuated. As it stands now, even the limits of the genus have become very uncertain. The present series of papers aims to present a taxonomic revision of the genus as well as a clarification of the generic concept concerning it.

The ovaricolous smuts of *Rhynchospora* reflect one of the chaotic phases of the taxonomy of *Cintractia.* *Ustilago montagnei,* the earliest species in this group of fungi, was described by Tulasne (10), based upon a collection from Algeria. Unfortunately this fungus has never been recollected from that area. Desmazières noted that the smut commonly found on *Rhynchospora alba* in France had spores larger than those of Tulasne's species and consequently proposed var. *major* to accommodate it. Hennings (4) created another epithet, *Ustilago taubertiana,* for a similar fungus from Brazil. Both species were later transferred from *Ustilago* to *Cintractia.* Clinton (2) separated *Cintractia taubertiana* from *C. montagnei* chiefly on the basis of spore size, but in a way contradictory to their original descriptions. Liro (9) suggested that the specific name *"montagnei"* should be avoided because Tulasne recorded the host as *Schoenus* and his original citation of spore size as 9–11 μ in diameter did not fit the forms occurring on *R. alba* in Europe. Accordingly he raised the variety *major* to specific rank and set up a new name, *Cintractia gigantospora,* for the European forms. In addition to the situation set forth above, a number of other binomials have been published from time to time for smuts occurring in the ovaries of *Rhynchospora* spp. Zundel

(11) and Hirschhorn (7) have each suggested that several of these species are synonymous, but both have failed to interpret them correctly.

In the present paper, six species and two varieties of *Cintractia* are recognized for the ovaricolous smuts of *Rhynchospora.* Descriptions and a key to species and varieties are presented. Only material actually studied is cited, and the institution in which each specimen so cited is to be found is designated by an abbreviation. These abbreviations are as follows: BPI = Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture; CH = Clinton Herbarium, Connecticut Agricultural Experiment Station; FH = Farlow Herbarium, Harvard University; K = Royal Botanic Gardens, Kew; NY = New York Botanical Garden; P = Museum National D'Histoire Naturelle, Paris; S = Naturhistoriska Riksmuseet, Stockholm.

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KEY TO SPECIES

False membrane of the sorus very inconspicuous, disappearing early
  Spores globose to oblong
    Spores smooth.......................................*C. amazonica*
    Spores indistinctly ornamented
      Spores pitted
        Spores 8–12 μ in length...........*C. montagnei* var. *minor*
        Spores 9.5–14 μ in length....................*C. montagnei*
        Spores 11–18 μ in length..........*C. montagnei* var. *major*
      Spores with short, sinuous striae...............*C. gigantospora*
    Spores coarsely tuberculate.......................*C. nova-guineae*
  Spores deeply cupped, appearing reniform in side view.......*C. farlowii*
False membrane of the sorus conspicuous and persistent.......*C. spicularum*

CINTRACTIA AMAZONICA Syd., Ann. Myc. **14**: 73. 1916.

Cintractia rhynchosporae Cif., Ark. Bot. **23A** (14): 9. 1931.

Sori in the ovaries, subglobose to ellipsoid, 1–2 mm. in length, each forming a firmly agglutinated spore mass, at later stages becoming rather dusty in the outer parts and more or less exposed between the spreading glumes. Spores smoky reddish brown, smooth, globose to broadly oval, occasionally slightly angular or oblong, 12.5–18 × 9–16.5 μ, rarely up to 21 μ in length, epispore 1–1.5 μ thick, evenly thickened.

Material examined: On *Rhynchospora barbata* (Vahl) Kunth (= *R. pterocarpa* Clarke). Brazil: Serra do Mel, Rio Branco, Aug. 1909, E. Ule 3345, *cotype* (S). Dominican Republic: Llano Costero, Prov. Santo Domingo, Sabana de Guerra, Aug. 13, 1929, E. L. Ekman 2515, *type* of *C. rhynchosporae* (BPI); Samana, Sabana de la Mar, July 4, 1937, C. E. Chardon 701 (BPI).

CINTRACTIA FARLOWII Clint. apud Zundel, N. Amer. Fl. **7**: 1000. 1939.

Sori in the ovaries, completely concealed by the glumes, 1–1.5 mm. in length; spore mass black, at first semi-agglutinate and rather compact, becoming dusty at maturity. Spores 9–12.5 μ in length, finely punctate, with epispore thinner and deeply compressed at one side, spore thus appearing reniform in side view, but broadly oblong in top view with a light brown, narrower portion in the middle and cap-like, deep cinnamon brown portions at both ends.

Material examined: On *Rhynchospora capitellata* (Michx.) Vahl (= *R. glomerata* Vahl var. *leptocarpa* Blake). United States: Fort Reed, Florida, S. Rapp (CH); Magnolia, Massachusetts, Sept. 27, 1911, Cora H. Clark, *type* (CH).

CINTRACTIA GIGANTOSPORA Liro, Ann. Acad. Sci. Fenn. A. **42**: 47. 1938.

Ustilago rhynchosporae Sauter, Mittheil. Gesellsch. Salzb. Landesk. **18**: 114. 1878.

Ustilago gigantospora Lehtola, Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo 17 (3): 23. 1942.

Sori in the ovaries, globoid, approximately 1 mm. diam., more or less exposed between the spreading glumes; spore mass black, usually remaining agglutinate and compact at maturity, surrounding a rudimentary columella. Spores globose to oval, occasionally angular, 14.5–21 × 10.5–18 μ, averaging around 17 × 15 μ; epispore deep reddish brown, 1–1.5 μ thick, under oil immersion lens appearing as ornamented with very short, sinuous, depressed striae, sometimes even as irregular and imperfect reticulations.

Material examined: On *Rhynchospora alba* (L.) Vahl. Austria: Salzburg, in Klotz. Herb. Viv. Myc. 1896, type of *U. rhynchosporae* (BPI); Edelbach pr. Windischgarsten, in Thuem. Fungi Aust. 337 (BPI). Finland: Alandia, Finström, Storträsk, in Liro, Myc. Fenn. 26, *type* (BPI). Sweden: Uppland, Möja, Fomby, Sneven, Aug. 2, 1918, T. Vestergren (S).

On *Rhynchospora miliacea* (Lam.) Gray. United States: Gainesville, Florida, June 17, 1938, E. West 12375 (BPI).

The collection on *Rhynchospora miliacea* is not typical of this species in having rather pulverulent sori at maturity and darker spores.

CINTRACTIA MONTAGNEI (Tul.) Magn., Abh. Bot. Ver. Prov. Brand. **37**: 79. 1896.

Ustilago montagnei Tul., Ann. Sci. Nat. Bot. III. **7**: 88. 1847.
Microbotryum montagnei Lév., Ann. Sci. Nat. Bot. III. **8**: 372. 1847.
Ustilago taubertiana P. Henn., in Engl. Bot. Jahrb. **17**: 525. 1893.
Ustilago psilocaryae Tr. & Earle, Bull. Torrey Bot. Club **26**: 493. 1899.
Cintractia taubertiana Clint., Jour. Myc. **8**: 142. 1902.
Cintractia psilocaryae Clint., Jour. Myc. **8**: 142. 1902.
Cintractia leveilleana R. Maire, Bull. Soc. Myc. Fr. **21**: 143. 1905.
Cintractia samanensis Cif., Ark. Bot. **23A** (14): 13. 1931.

Sori in the ovaries, inconspicuous, subgloboid to oval, 1–2 mm. in length, usually completely hidden by the enveloping glumes; spore mass black, compact at earlier stages, later the outer parts becoming dusty but the interior usually remaining firm and surrounding a rudimentary columella. Spores subglobose to oblong, frequently more or less angular, often laterally compressed on both sides, 9.5–14 × 8–13 μ, averaging around 11.5 μ in length, light to medium reddish brown, occasionally with smoky tint, finely to distinctly pitted, epispore 0.8–1.5 μ thick, usually unevenly thickened, frequently with remains of hyaline enveloping membrane attached.

Material examined: On *Rhynchospora alba* (L.) Vahl. France: S. Léger près Rambouillet (Seine et Oise), Aug. 25, 1850, Tulasne (P); Marais tourbeux des Moraux, in Rab. Fungi Eur. 2499 (BPI). Germany: Wussina, pr. Muskau, in Syd. Ustil. 180 (BPI); Aachen, in Rab. Fungi Eur. 283 (BPI); Eppendorfer, Hamburg, in Jaap, Fungi Sel. Exs. 13 (BPI). United States: Somerset County, Pennsylvania, May 15, 1936, H. W. Thurston (BPI).

On *Rhynchospora eximia* (Nees) Boeck. (= *Psilocarya scirpoides* Torr.). United States: Porter County, Indiana, Aug. 23, 1925, C. C. Deam (BPI).

On *Rhynchospora fauriae* Franch. Japan: Aomori, U. Faurie, *type* of *C. leveilleana.*

On *Rhynchospora glauca* Vahl (= *Schoenus laxus* Poir.). Algeria: La Calle, Aug. 2, 1841, Durieu du Maisonneuve, *type* (P).

On *Rhynchospora marisculus* Lindl. & Nees. Brazil: Clark Island, San Francisco, S. Catharina, Jan. 1885, E. Ule (FH); San Francisco, S. Catharina, E. Ule 1608 (CH).

On *Rhynchospora nitens* Gray (= *Psilocarya rhynchosporoides* Torr.). United States: Horn Islands, Mississippi, Oct. 1, 1898, S. M. Tracy, *type* of *U. psilocaryae* (BPI).

On *Rhynchospora oligantha* Gray. Dominican Republic: Cordillera Central, Samana, Sabana de la Mar, July 11, 1930, E. L. Ekman, *type* of *C. samanensis* (S).

On *Rhynchospora* sp. Brazil: San Francisco, S. Catharina, in Rab. Fungi Eur. 3806 (BPI).

The type of *Ustilago taubertiana* was not available for study. However, one of the Brazilian collections (Ule 1608) was recorded as such by Hennings (6).

Pro-ustilago rhynchosporae is a name applied by Brefeld (1) to a fungus on *Rhynchospora alba* morphologically identical with *Cintractia,* but producing lateral sporidia on the promycelium in germination. Since he did not describe its morphology, the name can be treated only as a *nomen dubium* or *nomen nudum.*

The collections assigned here to *C. montagnei* and its varieties show a great deal of variation in the size and color of spores, which measure from 8–11.5 μ long in a collection from Caucasus, U.S.S.R., on *Rhynchospora alba* to 12–18 μ long in the type of *Cintractia eximia* Cif. However, they can be grouped into three classes according to the spore size: (1) 8–12 μ long; (2) 9.5–14 μ long; and (3) 11–18 μ long. The color of spores becomes lighter as the spores decrease in size. These groups of course overlap and cannot be sharply delimited. These collections apparently all belong to one fluctuating natural group of biotypes. For convenience, the two extreme groups with spores smaller and larger than the type of *C. montagnei* are here treated as two varieties of this species.

Cintractia montagnei (Tul.) Magn. var. **minor** var. nov.

Sporis 8–12 × 6–10.5 μ, medio circa 10 × 8.8 μ, episporis pallide rubrobrunneis, circa 0.5 μ crassis; ceteris ut in specie.

Spores 8–12 × 6–10.5 μ, averaging around 10 × 8.8 μ, epispore light reddish brown, approximately 0.5 μ thick; otherwise identical with the species.

Material examined: On *Rhynchospora alba* (L.) Vahl. Czechoslovakia: in Kavina & Hilitzer, Crypt. Cech. Exs. 114 (BPI). Russia: Caucasus, Kobuleti, prope Batum, Sept. 9, 1917, Woronow, ex Herb. W. Siemaszko, **type** (BPI).

CINTRACTIA MONTAGNEI (Tul.) Magn. var. MAJOR Desm., Plant. Crypt. I. 1726. 1850.

Ustilago juncicola Speg., Rev. Argent. Hist. Nat. **1**: 170. 1891.
Ustilago scleriae (DC.) Tul. var. *dichronemae* P. Henn., in Engl. Bot. Jahrb. **17**: 526. 1893.
Ustilago liebmanni P. Henn., Hedwigia **33**: 229. 1894.
Ustilago dichronemae P. Henn., Hedwigia **43**: 78. 1904 (non *Ustilago dichronemae* P. Henn. on *Dichromena pubera* Vahl, 1904).
Ustilago bipustula Pk. apud House, N. Y. State Mus. Bull. **219–220**: 235. 1921.
Cintractia ekmani Cif., Ark. Bot. **23A** (14): 11. 1931.
Cintractia eximia Cif., Ark. Bot. **23A** (14): 12. 1931.
Cintractia major Liro, Ann. Acad. Sci. Fenn. A. **42**: 46. 1938.
Cintractia juncicola Liro, Ann. Acad. Sci. Fenn. A. **42**: 286. 1938.
Ustilago intercedens Lehtola, Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo **17** (3): 23. 1942.
Cintractia standleyana Zundel, Mycologia **35**: 171. 1943.

Spores frequently angular, 11–16.5 × 7.5–14.5 μ, mostly averaging around 14 × 11 μ, the most elongate up to 18 μ in length, epispores deep reddish brown, usually with smoky tint, 1–1.5 μ thick; otherwise identical with the species.

Material examined: On *Rhynchospora alba* (L.) Vahl. Austria: Egelsee nächst Millslatt, Kärnsten, in Weese, Eumyc. Sel. Exs. 298 (BPI). Finland: Isthmus Karelicus, Raibola, in Liro, Myc. Fenn. 27 (BPI). France: in Desm. Pl. Crypt. Fr. 2126 (BPI). Germany: Siegburg, in Thuem. Myc. Univ. 929 (BPI);

Siegburg, environs de Bonn, in Fckl. Herb. Barbey-Boiss. 1836 (BPI). United States: York, Maine, in Seym. & Earle, Econ. Fungi C7 (BPI); Eastern Texas, 1888, G. C. Neally, sub *Ustilago rhynchosporae* Ell. & Galw. (BPI).

On *Rhynchospora ciliaris* (Michx.) Mohr (= *R. ciliata* Vahl). United States: De Funiak Springs, Florida, Sept. 30, 1903, M. B. Stevens (CH).

On *Rhynchospora distans* (Michx.) Vahl. Bermuda: Devonshire Marsh, Aug. 8, 1921, H. H. Whetzel (BPI).

On *Rhynchospora eximia* (Nees) Boeck. Dominican Republic: Cordillera Central, prov. Azua, El Tetero, Oct. 10, 1929, E. L. Ekman, *type* of *C. eximia* (S).

On *Rhynchospora glauca* Vahl. Guatemala: Dept. Alta Verapaz, Apr. 14, 1941, P. C. Standley, *type* of *C. standleyana* (BPI).

On *Rhynchospora globularis* (Chapm.) Small var. *recognita* Gale. United States: Lanham, Maryland, July 21, 1906, A. Chase (CH).

On *Rhynchospora glomerata* Vahl. United States: Selbyville, Delaware, in Barthol. Fungi Columb. 3008 (BPI).

On *Rhynchospora marisculus* Lindl. & Nees. Dominican Republic: Cordillera Septentrional, prov. Puerto Plata, Cabarete, in Cif. Mycofl. Doming. Exs. 84, sub *Cintractia portus-argenti* Cif. (BPI, S).

On *Rhynchospora nana* (Boeck.) Pfeiff. (= *Dichromena minarum* C. B. Clarke). Brazil: Glaziou 20045, *type* of *U. dichronemae* (CH).

On *Rhynchospora odorata* Wright (= *R. stipitata* Chapm.). Bermuda: Devonshire Marsh, Mar. 14, 1922, H. H. Whetzel (BPI).

On *Rhynchospora podosperma* Wright. Dominican Republic: Llano Costero, prov. Santo Domingo, Cuenca, in Cif. Mycofl. Doming. Exs. 8, *type* of *C. ekmani* (BPI).

On *Rhynchospora* sp. Mexico: Mirador, Liebmann 691, *type* of *U. liebmanni* (CH). Paraguay: Vallée de l'Y-acan-Guazu, in Balansa, Pl. Parag. 4323 (BPI, FH).

On *Rhynchospora tenuis* Link. Honduras: Dept. Morazán, Quebrada de Santa Clara, Dec. 17, 1946, P. C. Standley & L. O. Williams (BPI).

The type of *Ustilago juncicola* is not found in the Instituto de Botanica "Spegazzini," La Plata. In B. Balansa's Pl. du Paraguay are included two collections, nos. 4294 and 4323, labelled as such, the latter being cited originally by Spegazzini. No. 4294 at the Farlow Herbarium contains some plants of *Fimbristylis* infected by *Cintractia axicola* (Berk.) Cornu. No. 4323 at the Mycological Collections, Bureau of Plant Industry is merely *Cintractia montagnei* var. *major,* while the same number at the Farlow Herbarium consists of a mixture of *C. montagnei* var. *major* and *C. axicola.* Since Spegazzini's description fits better to *C. montagnei* var. *major,* the name *U. juncicola* is included here as a synonym.

Ustilago liebmanni was originally described as on *Luzula.* Clinton (3) considered it as a synonym of *Cintractia junci* Trel. in accordance with the suggestion of Hennings himself. An examination of its type, however, disclosed that it is identical with *C. montagnei* var. *major* and its host is a *Rhynchospora.*

Cintractia standleyana was described as a new species chiefly on the ground that the infected flowers become aggregated capitately as a result of the shortening of pedicels. On certain hosts, however, it is not unusual for this group of fungi to produce a witches'-broom effect in various degrees. This characteristic alone is hardly sufficient to separate species.

CINTRACTIA NOVA-GUINEAE Zundel, Mycologia 31: 589. 1939.

Sori in the ovaries, completely hidden by the glumes, 2–3 mm. long; infected flowers becoming aggregated as a result of the shortening of pedicels. Spores when mature dark reddish brown, opaque, subglobose to ellipsoidal, often somewhat irregular, variable in size, ranging 16.5–29 μ long, densely covered by coarse, blunt tubercles measuring 1–1.5 μ wide at base, appearing irregularly reticulate on top.

Material examined: On *Rhynchospora* sp. New Guinea: Marsh Meadows, Morobe, Dec. 22, 1938, M. S. Clemens, *type* (BPI).

Zundel stated that this species is closely related to *Cintractia amazonica* but differs from it in having minutely pitted spores, while the spores of *C. amazonica* are verruculate. On the contrary, *C. amazonica* has perfectly smooth spores, while the spores of the present species are prominently ornamented.

CINTRACTIA SPICULARUM Racib., Bull. Intern. Acad. Sci. Krakow **1909**: 353. 1909.

Cintractia axicola (Berk.) Cornu f. *spicularum* Juel, Bih. till K. Sv. Vet.-Akad. Handl. **23** (10): 7. 1897.

Sori destroying the ovaries, ovoid, cornute or narrowly ellipsoid, tapering at the apices, 3–7 mm. in length, each covered by a conspicuous, whitish, tough false membrane which persists even after its rupture; spore mass black, agglutinate, later becoming dusty on the outer parts, but usually remaining compact in the basal part of the sorus, surrounding a very short, slender columella. Spores chiefly globose to oval, often compressed, medium to deep reddish brown, often with smoky tint, 12–16.5 μ in length, epispore smooth, about 1 μ thick.

Material examined: On *Rhynchospora corymbosa* (L.) Britton (= *R. aurea* Vahl). British Guiana: Pl. Vryheid, Feb. 14, 1924, D. H. Linder 941 (BPI). Dominican Republic: El Valle, Samaná, July 4, 1937, C. E. Chardon (BPI). India: C. B. Clarke, sub *Ustilago conglobata* Cke. & Massee (K, NY). Indo-China: Cho-Ganh, Tonkin, Oct. 1922, A. Pételot (BPI); Tourane and vicinity, May–July, 1927, J. & M. S. Clemens 3978 (FH). Java: Sockanegara, Preanger, M. Raciborski (FH). Mexico: Coatzacoalcos, Jan. 1895, C. L. Smith (BPI); Jalapa, in Syd. Ustil. 220 (BPI). Philippine Islands: Zamboanga, Mindanao, Nov.–Dec. 1911, E. D. Merrill 8322 (BPI). Puerto Rico: Mayaguez, in Seym. & Earle, Econ. Fungi C105 (BPI); vicinity of Vega Baja, March 31, 1922, N. C. Britton *et al.* 6967 (CH). Trinidad: Border of Pitch Lake, March 27, 1920, N. L. Britton *et al.* (BPI).

The host of one of the Puerto Rican collections (Britton *et al.* 6967) cited above was erroneously labelled as *Mariscus jamaicensis*, which was also recorded by Zundel (11).

Cintractia utriculicola (P. Henn.) Clint. is the name commonly applied to this fungus. While describing *Cintractia axicola* (Berk.) Cornu f. *spicularum*, Juel (8) stated that his fungus was undoubtedly identical with *Cintractia leucoderma* (Berk.) P. Henn. f. *utriculicola* P. Henn. Clinton (2) accordingly raised Hennings' form to specific rank and cited Juel's form as its synonym. In so doing, he based his decision only on an exsiccati specimen issued in Sydow's Ustilagineen no. 220 as *C. axicola* f. *spicularum.* A review of Hennings' original note on *C. leucoderma* f. *utriculicola,* however, revealed that the concept of Juel, Clinton, and later authors regarding the identity of the two fungi mentioned above is incorrect. Hennings' (5) writing is translated as follows:

> *Cintractia leucoderma* (Berk.) P. Henn. = *C. Krugiana* P. Magn. Brazil: S. Cathar. at Blumenau in *Rhynchospora gigantea* n. 293.
>
> This species was placed correctly by P. Magnus in *Cintractia* but he incidentally overlooked the fact that the same fungus had been described by Berkeley as *Ustilago leucoderma.* The same species also occurs on *Rhynchospora aurea* in Ratnapoora, as well as in Borneo, and also in Usambara, where it was collected by Dr. Stuhlmann. Unfortunately I have named the fungus originating from the latter place as *Cintractia Krugiana* P. Magn. var. *usambarensis* in Engler's Pflanzenwelt Ostafrikas, so that I correct the error herewith. The form of the sori and the spores is somewhat variable. —In addition to its occurrence on the axes of the inflorescences the fungus is also found inside the utriculi, which rupture like blisters, and the often spherical sori are each covered at first by a thick white-shining membrane. It is characteristic that while in such specimens the fungus never occurs in the axes; on the other hand in specimens in which the axes are attacked and swollen cylindrically, the flowers are either entirely stunted or when developed are without smut pustules.—The spores appear to me in both cases entirely identical. The form which occurs only in the utriculus was collected by P. Sintenis from Puerto Rico as well as by Dr. A. Möller and by E. Ule at Blumenau at the same localities on *Rhynchospora gigantea* with the typical form. I designate these as form. *utriculicola,* while Schröter places them in his herbarium under *Ustilago Caricis* (Pers., Fuck. = *Cintractia C.* P. Magnus).

Despite the fact that the utriculus does not exist in *Rhynchospora,* Hennings apparently referred to forma *utriculicola* a form similar to the typical *C. leucoderma* in the blister-like pustules, the white and thick enveloping membranes, and the characters of the spores. On certain species of *Rhynchospora, C. leucoderma* or related forms frequently infect the secondary pedicels of the cymes, form short, oblong to oval or even globoid spore masses below the rudimentary abortive spikelets, and often are embraced partially by the bracts. Sometimes the infection even extends to the scales. In that way, the sori may appear as if occurring inside the florets. Hennings most probably referred his forma *utriculicola* to this sort of infection instead of any of the other ovary-attacking fungi which can be readily distinguished from *C. leucoderma.*

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AN ADDITION TO THE MYXOMYCETE GENUS COMATRICHA

R. K. Benjamin and A. W. Poitras

(WITH 4 FIGURES)

The genus *Comatricha,* according to the most recent monograph by Martin (4), is represented in North America by twenty-one species. Two European species, *Comatricha filamentosa* and *C. fragilis,* described by Meylan (5, 6), are as yet unknown from this continent. Species of this genus of the Stemonitaceae are characterized by having globose to cylindrical, usually stipitate sporangia, possessing an evanescent, rarely persistent peridium, and in most species, with a definite columella from which the capillitium arises. The capillitium is composed, typically, of a network of branching and anastomosing threads, the extremities of which are free. The spores are dark colored, usually ferruginous, violaceous, or black.

During the past two years the senior author has made numerous collections of Myxomycetes in Illinois; * included are eleven species of *Comatricha: C. aequalis* Peck, *C. cornea* G. Lister & Cran, *C. elegans* (Racib.) Lister, *C. fimbriata* G. Lister & Cran, *C. irregularis* Rex, *C. laxa* Rost., *C. longa* Peck, *C. nigra* (Pers.) Schroet., *C. pulchella* (Bab.) Rost., *C. rubens* Lister, and *C. typhoides* (Bull.) Rost.

Recently an old, dry, sample of goat dung, originally collected and moistened in November 1947, was moistened again for the reisolation of mucoraceous fungi. On subsequent examination, a large number of sporangia of a *Comatricha* were obtained which we have been unable to assign to any of the previously described species of the genus.

The extensive collections of Myxomycetes in the Herbarium of The State University of Iowa have been examined in order that our specimens might be compared with North American and foreign species not previously seen by the authors. We are indebted to Professor G. W. Martin for his assistance in making available for study this collection of Myxomycetes, and for examining our material. Because this species of *Comatricha* is so clearly distinct from known species of the genus, we feel it merits description as new. The specific epithet *mirabilis* has been selected because of the beauty of the sporangia and the striking pattern of the reticulate spores.

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Comatricha mirabilis sp. nov.*

Sporangiis solitariis vel gregariis, erectis, intense brunneis, ellipsoideis vel cylindraceis, 0.7–1.5 mm. altis, 0.25–0.35 mm. diam.; stipitatis; stipite intensius brunneo, 0.35–0.75 mm. alto, in capillitium tamquam columella penetrante et vix apicem attingente; capillitio simplice vel parce ramoso, e tota columella enascente; sporis globosis, intense violaceis-brunneis, plane reticulatiis, 10–13 μ diam.

Sporangia bone-brown, in small clusters, erect, short-cylindrical to ovoid, 0.7–1.5 mm. tall, 0.25–0.35 mm. in diam., stipitate; peridium very evanescent, persistent at the base as a small collar; hypothallus conspicuous, brown, more or less common to a cluster of sporangia; stipe about half the total height, brownish-black, continuing into the sporangium as a columella which reaches nearly to the apex; capillitium arising from the entire length of the columella, consisting of simple or dichotomous, rarely anastomosing, purple-black threads, slender and paler near the base, gradually expanding outwards and ending in short, much thickened, rigid, diverging branchlets which project beyond the surface of the sporangium; spores dark lilac-brown, reticulate with narrow raised bands, about 20–30 meshes to the hemisphere, 10–13 μ in diam. (av. 11.1 μ) including the border which is 1–2 μ high (av. 1.4 μ).

The type appeared on goat dung collected near Urbana, Champaign County, Illinois, November 1947 (moist chamber culture), and has been deposited in the Mycological Collections, University of Illinois Herbarium (Mycological Collections No. 21003). Isotypes have been sent to the Mycological Collections of the Bureau of Plant Industry, Beltsville, Md.; Farlow Herbarium, Harvard

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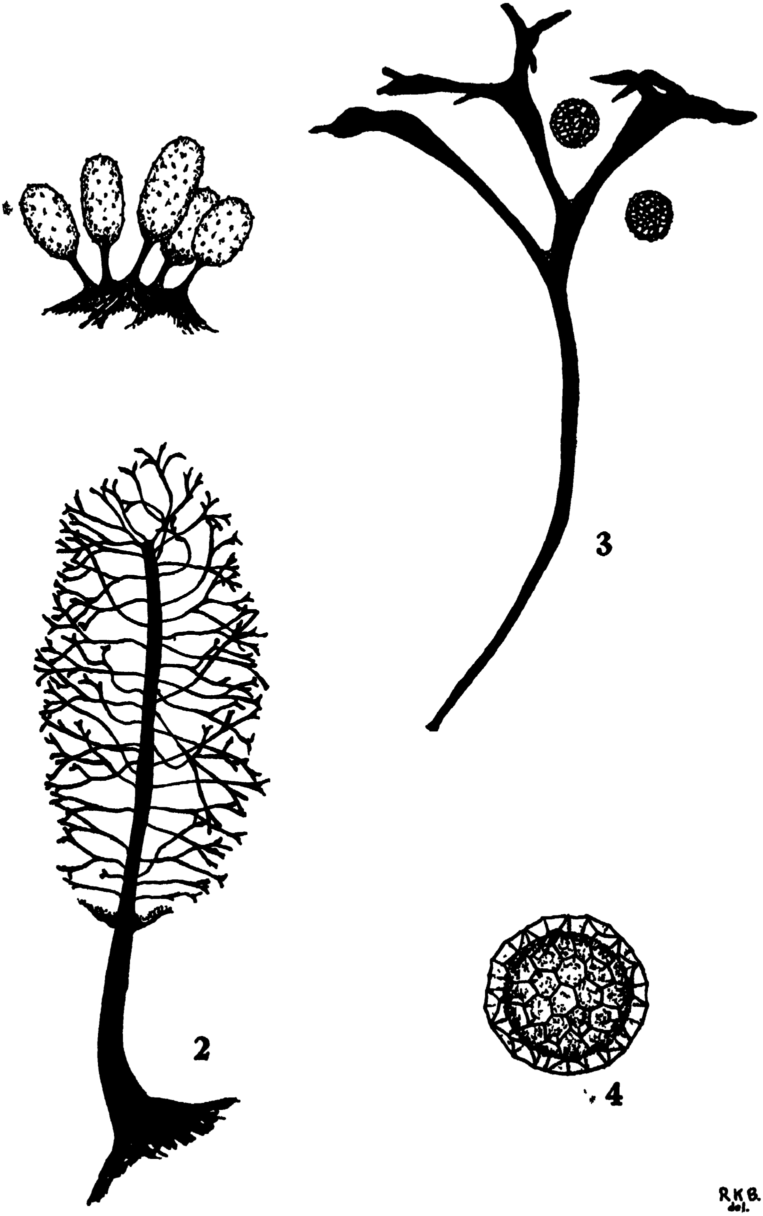
* The authors wish to thank Professor John L. Heller, Head of the Department of Classics, for checking the Latin diagnosis.



FIGS. 1–4. *Comatricha mirabilis.*

University; the Herbarium of the New York Botanical Garden; and the Herbarium of the State University of Iowa, Iowa City.

Comatricha mirabilis is readily distinguished from all other previously described species of *Comatricha* by having a simple capillitium and spores which are strongly reticulate with narrow raised bands. The capillitium bears a marked resemblance to that of *Comatricha fimbriata* G. Lister & Cran (2) in its simplicity and general structure, but the reticulate spores of *C. mirabilis* are distinctly different from the verrucose spores of *C. fimbriata,* a species we have collected frequently on bark. One other species of *Comatricha, C. rispaudii* Hagelstein (1), is known which produces conspicuously banded reticulate spores. This differs from *C. mirabilis,* however, in that it possesses a more netted capillitium and has spores which are smaller, measuring 9 μ or less in diameter. *Comatricha reticulata* H. C. Gilbert (7) and *C. cylindrica* (Bilgram) Macbr. (3) produce spores which are more or less reticulate, but in these species the ridges are only slightly raised above the surface of the spore. Type material of both *Comatricha reticulata* and *C. rispaudii* has been studied for comparison. Authentically determined specimens of *Comatricha cylindrica* also have been examined.

Because of its small size, *Comatricha mirabilis* is doubtless very inconspicuous in nature. The plasmodium may develop in humus or soil, arising to fruit on grass or other herbaceous material. A number of species of Myxomycetes (i.e. *Physarum cinereum, Didymium squamulosum,* etc.) often appear on animal dung, but are more commonly found on dead leaves, grass, and other herbaceous ground cover.

The material here examined is sufficiently abundant and distinctive to indicate a normal development. The spores are perfectly mature and quite uniform in size and surface markings.

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EXPLANATION OF FIGURES

FIG. 1. Habit sketch of cluster of sporangia showing tips of capillitial branches projecting from surface. × 15. FIG. 2. Mature sporangium, after spores have been removed, showing nature of capillitium and columella. × 83. FIG. 3. Single capillitial strand and two spores. × 530. FIG. 4. A spore. × 1730.

TREATMENT OF ALLOMYCES JAVANICUS VAR. JAPONENSIS INDOH WITH COLCHICINE AND SODIUM NUCLEATE

E. S. Beneke and G. B. Wilson

Experimental treatment of higher fungi, especially Ascomycetes and Deuteromycetes, with colchicine and other chemicals has been reported by a number of workers [Richards (9); Vanderwalle (4); Steinberg and Thom (12, 13); Bauch (1, 2, 3); and Gordon and McKechnie (6)]. In some cases positive results have been obtained which have usually been ascribed to polyploidy or chromosomal aberrations. However, polyploidy has not generally been cytologically confirmed. Insofar as we are aware no similar treatments of any of the Phycomycetes have been reported. In view of the importance of the effect of these "mitotic poisons" on higher plants it appears desirable to study the effects of such chemicals on some of the lower fungi. The present note is concerned with our preliminary investigations in this direction. *Allomyces* was chosen because its life cycle apparently resembles alternation of generations in higher plants. Induced changes in the life cycle of this fungus, in addition to the studies of Sörgel (10, 11), Emerson (4), and Emerson and Wilson (5), should give further support to Kniep's (8) hypothesis that meiosis normally occurs in the resistant sporangia of *Euallomyces.*

The typical life cycle of *Euallomyces* is as follows: the sporophyte gives rise to two types of sporangia, the zoosporangia, giving rise to zoospores which form new asexual thalli; and the thick-walled resistant sporangia, giving rise to zoospores which develop into sexual thalli. Male and female gametangia are formed on the sexual thalli. These gametangia produce motile male and female gametes which fuse with each other and develop into the asexual thalli.

A number of variations in the "normal" life cycle have been reported. Female gametes have been observed occasionally to develop parthenogenetically [Kniep (8), Sörgel (10), and Emerson (4)]. Asexual thalli may be formed by the planont from resistant sporangia of certain strains [Sörgel (10), and Emerson (4)]. Sexual thalli have been noted to produce what are apparently resistant sporangia [Hatch (7), Sörgel (10), and Emerson (4)].

Method of Culture. The dried resting sporangia and germinating zygotes were put in Petri dishes containing either sterile distilled water, solutions of colchicine or of sodium nucleate. Portions of hemp seeds were placed in contact with either the dried resting sporangia or the germinating zygotes. The gametophytic or sporophytic thalli develop on the hemp seeds. Sterile distilled water was used to replace the colchicine or sodium nucleate solutions after a designated period of time. The mature sporophytic thalli with resting sporangia were dried on filter paper.

Treatments and Results: 1. *First treatment.* Germinating zygotes of a strain of *A. javanicus* var. *japonensis,* which normally has been producing only gametophytes from the resting sporangia, were treated with 1% sodium nucleate on February 25, 1949. Germinating zygotes were also grown as controls. The zygotes were allowed to develop in the solution for 65 hours and then were transferred to distilled water. The resulting sporophytes showed no obvious morphological changes. Additional colonies were formed from zoospores arising from these asexual thalli. The colonies from the treated and untreated zygotes were subsequently dried on filter paper for at least three weeks.

2. *Second treatment.* Sixteen of the dried colonies from the sodium nucleate treatment with numerous resistant sporangia were used for a colchicine experiment; five of them were placed in .01% colchicine, five in .02%, and six were cultured in distilled water as controls. In addition, sixteen previously untreated dried colonies of resting sporangia from the original strain were also used. Five were placed in .01% colchicine, five in .02%, and six were cultured as controls.

Within one day many sexual and asexual thalli were developing from the germination of zoospores from resistant sporangia which were derived from the sodium nucleate treated material. Only sexual thalli were produced by the germination of zoospores from

TABLE I

	.01% Colchicine			.02% Colchicine			No treatment		
	G & S	G	None	G & S	G	None	G & S	G	None
Sodium nucleate	5	–	–	1	4	–	3	3	–
No treatment	–	4	1	–	3	2	–	6	–

Note: Numbers refer to the number of colonies, each of which contain many resting sporangia.
G & S = Colonies having both Gametophytes and Sporophytes.
G = Colonies having Gametophytes only.
None = No germination of the resting sporangia.

resistant sporangia which had not previously been treated. The results of these treatments are shown in table I. At this time there was no indication of any direct effect of the colchicine.

3. *Third treatment.* Resting sporangia that were developed after the second treatment with .01% colchicine, .02% colchicine, or no treatment as listed in table I were dried for eight weeks. These resting sporangia from 54 dried colonies were germinated in distilled water with the results indicated in table II.

The results given in table I and table II show that treatment with both sodium nucleate and colchicine is correlated with an increase in the production of sporophytes from the resting sporangia.

Discussion. Production of mixed thalli, as noted above, has been reported. There is no doubt from the present data that sodium nucleate treatment of germinating zygotes has increased the frequency with which resistant sporangia from the sporophyte generation develop mixed thalli. It seems likely that colchicine has a similar effect. The explanation of this difference is not readily apparent and the matter deserves considerable further investigation. As a tentative working hypothesis we suggest the possibility

TABLE II

First treatment	Second treatment	G & S	G	None
No treatment	No treatment	—	9	—
Sodium nucleate	No treatment	—	6	—
No treatment	.01% Colchicine	—	12	—
No treatment	.02% Colchicine	3	—	3
Sodium nucleate	.01% Colchicine	—	12	3
Sodium nucleate	.02% Colchicine	3	3	—

that: (a) haploid thalli are inherently "gametophytic" and that diploid or polyploid thalli are inherently "sporophytic." (b) Treatment of the germinating zygote induces some nuclei to become polyploid and thus the resulting asexual thalli may be mixoploids. (c) At reduction division during the germination of the resistant sporangia some planonts would be haploid and some diploid or polyploid. The haploid would then be expected to give rise to sexual thalli, and the others to asexual thalli. This hypothesis remains to be verified by extensive cytological investigations.

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ASCOCHYTA LEAF SPOTS OF CEREALS AND GRASSES IN THE UNITED STATES[1]

RODERICK SPRAGUE AND A. G. JOHNSON[2]

(WITH 2 FIGURES)

In the course of studies of sphaeropsidaceous leaf spots on Gramineae, the need has been felt for an analysis of the genus *Ascochyta* as it occurs on the grass family. Information on species of *Ascochyta* on these hosts in the United States has not been assembled previously. Except for the article by Davis (**11**), information is scanty. The writers have encountered unreported species and a number of collections have extended the host range as well as the geographic range of known species. Some of these data have been reported (**50, 51**).

A study of the genus *Ascochyta* is made somewhat difficult because it seems to include not only species with well fixed characters, but also virtually identical material that is only a transitory phase of certain fungi in related genera. In many cases, spores that look like those of *Phyllosticta, Ascochyta, Stagonospora,* short-spored *Septoria,* and even *Hendersonia* all may occur in the same pycnidium or in close proximity. Therefore, these *Ascochyta*-like phases may not be readily distinguished in all instances from true species of *Ascochyta* that may rarely or never develop spores with more than one septum and which are genetically distinct from the facsimiles. For example, the 1-septate phase of *Septoria nodorum* Berk. on material collected early in the season is especially confusing. The spores of this species, however, have slightly truncate bases and usually appear more slender than those of *A. sorghi* (*A. graminicola*). Also, at least a few 3-septate spores, characteristic of *S. nodorum*, may be found if careful examination is made with the aid of stains such as dilute cotton blue. As the spores mature, the 3-septate condition becomes more common. Furthermore, growing the fungi in pure culture also may aid in distinguishing them, as species of *Hendersonia* more often have mouse-colored, olivaceous or dark mycelia while species of *Ascochyta* and *Stagonospora* tend to have buff, light gray, or white, cottony mycelial colonies. The species of *Septoria* with filiform spores, on the other hand, have mucose, later carbonaceous or subcottony colonies. These characters are not strictly fixed but have proved of some value in classifying the group.

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[1] Cooperative investigations between the Divisions of Cereal Crops and Diseases; Forage Crops and Diseases; and Soil Management and Irrigation, Bureau of Plant Industry, Soils, and Agricultural Engineering and the Nursery Division, Soil Conservation Service, U. S. Dept. of Agriculture and the Oregon, Washington, and North Dakota Agr. Exp. Stations. Published with the approval of the Director of the Oregon Exp. Sta. as Tech. Paper No. 401. Contribution from the Dept. of Plant Pathology. Published with the approval of the Director of the Washington Agric. Expt. Sta. as Scientific Paper No. 869.

[2] Pathologist, Washington Agricultural Experiment Station, and collaborator, formerly principal pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils and Agr. Engineering, respectively. The writers acknowledge with sincere appreciation the many helpful suggestions received from Mr. J. A. Stevenson and Miss Edith K. Cash, Division of Mycology and Disease Survey. They wish to extend special thanks to Editor Alexander H. Smith for aid in the editing of the manuscript.

THE GENUS ASCOCHYTA

The genus *Ascochyta* was described in 1830 by Mlle. Libert (27, p. 8) who regarded the spores as minute asci and the cell contents as globose spores.

The spelling of the name of the genus was changed to *Ascochyta* apparently first by Link (30) in 1833. This revised spelling was followed by Mlle. Libert in 1837 (28) and by others since that time.

Mlle. Libert's interpretation of the spores as asci and the cell contents as globose spores was followed by Link (30) in 1833, Corda (10) in 1842, Rabenhorst (36) in 1844, and Bonorden (6) in 1851. Léveillé (26), however, in 1849, regarded them as spores and described them as oval, linear, simple or septate. In 1875, Saccardo (37, p. 302), in a brief footnote, emended the genus as follows:

> "*Ascochyta:* spermatiis (v. stylosporis) ovoideis v. oblongis, 1 septatis."

In 1878, Saccardo (38, p. 161) gave a condensed description, which emended the genus still further and in 1884 Saccardo (42, v. 3, p. 384) delineated the genus as it is recognized today.

It is this basic description that Saccardo (42, v. 10, 11, 14, 16, 18, 22, 25) and other authors, such as Allescher (2), Lindau (29), Davis (11) and Clements & Shear (9), have followed since that time. This still seems to be the preferable course to follow.

Some authors, however, have followed a different treatment. Tassi (53), in 1902, proposed several new genera in Sphaeropsidaceae for various intergrading forms. Among these was *Ascochytella,* erected to cover species with small, 2-celled, colored pycnospores. This included species of *Ascochyta* with slightly colored spores, as well as certain species of *Diplodia.* Saccardo did not recognize *Ascochytella* as a genus but, in 1906 and again in 1913 (42, v. 18, 22), used the name for one of his two sections of the genus *Ascochyta* as follows:

> "I. *Eu-Ascochyta.* Sporulae hyalinae.
> II. *Ascochytella* Fl. Tassi (ut gen.). Sporulae dilute coloratae."

Potebnia (34, p. 10), in 1907, suggested the name *Ascochytula* as a sub-genus under *Ascochyta* to include species with slightly colored pycnospores. Diedicke (12), in 1912 and again (13) in 1915, used both *Ascochytella* and *Ascochytula* as generic names for species with light brown spores and separated them by differences in the peridia and shape of the spores (pseudopycnidial peridia and light brown spindle-shaped spores in *Ascochytella* and complete parenchymatous peridia and light brown, round-ended spores in *Ascochytula*). Diedicke, both in 1912 and 1915, included in *Ascochyta* only species with hyaline spores. Davis (11), in 1919, included both hyaline and slightly colored spored species in the genus *Ascochyta* and did not use the genera *Ascochytella* or *Ascochytula.* Hoehnel (22, p. 316), in 1923, followed Diedicke both in including only species with hyaline spores in *Ascochyta* and in using *Ascochytella* and *Ascochytula* for species with light brown spores. He also separated the two genera by essentially the same characters of the peridia used by Diedicke. He, however, wrongly credited both genera to Diedicke. The tide turned, however, when Petrak (31), in 1925, indicated that these attempted distinctions did not have the slightest value, as such differences in pycnidial walls may occur within a species. Likewise, Grove (**19**, p. 328), in 1935, stated that "there is no real basis for the separation of some of the species of this genus [*Ascochytula*] under the name *Ascochytella* Died. on the ground that they have a thinner (pseudopycnidial) peridium." Curiously enough, however, Grove recognized the genus, *Ascochytula,* in preference to the older name *Ascochytella.* While the name *Ascochytula* was suggested by Potebnia (**34**) in 1907 as a sub-genus under *Ascochyta,* it was not raised to generic rank until Diedicke (**12**) did so in 1912. The genus *Ascochytella,* on the other hand, was erected by Tassi (**55**) in 1902. Furthermore, Clements and Shear (**9**, p. 363), in 1931, placed both *Ascochytella* and *Ascochytula* as synonyms under the genus *Ascochyta.* While Trotter (**59**), also in 1931, did not recognize either genus, he used both names for two of his sections of the genus *Ascochyta,* using essentially the same characters for the sections as employed by Diedicke for the genera, as follows:

> "I. *Eu-Ascochyta.* Sporulae hyalinae.
> II. *Ascochytella* (Tass.) Died., Ann. Myc. 10, 1912, p. 141.—Pseudopycnidiacea, maculicola, sporulis utrinque subacutatis subfusoideis, pallide brunneis.
> III. *Ascochytula* (Pot.) Died., Ann. Myc. 10, 1912, p. 141.—Pycnidia phomatoidea, parietibus crassis; sporulae pallide brunneae utrinque rotundatae."

Other related genera to be considered in this connection are *Stagonosporopsis* Diedicke, *Diplodina* Westendorp, and *Apiocarpella* Syd. (*Apiosporella* Speg.).

Stagonosporopsis.—The genus *Stagonosporopsis* was very sparingly described by Diedicke (**12**) in 1912. In 1915, he (**13**, p. 397) elaborated the description to include a fungus intermediate in morphology between *Ascochyta* and *Stagonospora.*

In 1923, Hoehnel (**22**) recognized *Stagonosporopsis* essentially by the same characters used by Diedicke. Petrak (**31**, p. 5), however, in 1925, in reviewing Hoehnel's (**22**) paper, stated that it was the same as *Ascochyta* and in 1943 (**31**) related it to *Stagonospora.*

Trotter (**59**) in 1931, however, recognized the genus. He adds, however, "Gen. *Ascochytae proximum.*" Clements and Shear (9), also in 1931, placed *Stagonosporopsis* Died. as a synonym under *Ascochyta.* Grove (19), in 1935, and Buchwald (8), in 1939, both recognize the genus. Buchwald, however, adds: "Sprague anerkender ikke Slaegten." (Transl. "Sprague does not recognize the genus.")

Ainsworth and Bisby (1), in 1943, and again in 1945, stated:

> "*Stagonosporopsis* Died. = *Ascochyta* (Sphaeropsid.) fide Petrak."

Diplodina.—The genus *Diplodina* was described by Westendorp (63, p. 562), in 1857.

Diplodina salicis, the type, was described as having hyaline fusiform spores with a single, central septum, and thus illustrated. In 1884, Saccardo (42, v. 3) assembled the characters of the genus *Diplodina* as follows:

> "Perithecia subcutanea vel erumpentia, globose-papillate, atra glabrescentia. Sporulae ellipsoideo-oblongae, 1-septatae, hyalinae.—Est *Diplodia* hyalodidymia."

It is this basic description of the genus *Diplodina* that Saccardo followed subsequently; likewise Lindau (29) in 1900. Allescher (2), also in 1900, followed the same description for the genus but tried to limit it to species on branches and stems and admitted that it was next to impossible to separate the genus from *Ascochyta* by morphological characters of the pycnidia.

In 1902, Tassi (55) proposed a rather elaborate revolutionary system for these related genera. For the *Hyalodidymae,* he proposed (1) restricting *Ascochyta* to species with 1-septate, hyaline spores up to 15 μ long and borne on leaves; (2) a new genus, *Diplodinula,* with similar spores on stems; and (3) restricting *Diplodina* to species with hyaline, 1-septate spores more than 15 μ long, apparently regardless as to whether on stems or leaves. Saccardo (42, v. 18), in 1906, disagreed with Tassi, but divided *Diplodina* into two sections as follows:

> "I. *Eu-Diplodina* (*Diplodinula* Fl. Tassi). Species microsporae.
> II. *Diplodaria.* Species macrosporae; sporulae supra 15 μ longae."

Diedicke (12), in 1912, separated *Ascochyta* and *Diplodina* on the basis of whether the pycnidia were on leaves (*Ascochyta*) or stems (*Diplodina*). In 1915, Diedicke (13) separated these two genera by essentially similar characters. Hoehnel (22), in 1923, used *Diplodina* in the inclusive sense essentially in the same manner as Saccardo (42, v. 3, p. 411), in 1884, except that he specified that the two cells of the spores be equal ("gleich-zweizellig"), and referred species with very unequal cells ("sehr ungleich-zweizellig") to the genus *Apiosporella* Speg. Petrak (31), in 1925, in reviewing Hoehnel's paper, again objected as follows:

> "*Diplodina* in Sinne der meisten Autoren wird neben Ascochyta nicht aufrecht zu halten sein."

Buchwald (8), however, in 1939, separated *Diplodina* from *Ascochyta* by essentially the same characters used by Diedicke (13).

Apiocarpella.—Spegazzini (45), in 1910, named a sphaeropsidaceous genus *Apiosporella* and described it as follows:

> "Char. *notis externis* Ascochytae; sporulis excentrice septatis, loculo supero maximo infero minuto appendiculiformi, hyalinis."

Sydow, H. and P. (54), in 1919, however, substituted the name *Apiocarpella* for *Apiosporella* Speg. as the latter genus name was preoccupied by *Apiosporella* Hoehn., an ascomycetous genus. While Clements and Shear (9), in 1931, placed *Apiosporella* and *Apiocarpella* as synonyms under *Ascochyta*, Trotter (59), also in 1931, Wehmeyer (62), in 1946, and Sprague (52), in 1948, recognized the genus *Apiocarpella* as distinct from *Ascochyta*. This seems justifiable especially because of the eccentric septum and the resulting unequal size of the cells of the spores.

✓*General considerations.*—With everything considered, the writers favor Saccardo's basic concept of the genus *Ascochyta* Lib., as given in 1884 (42, v. 3, p. 384), with the further specification that the 1-septate spores have the septum approximately centrally located. This permits the recognition of *Apiocarpella* Syd. as a valid genus. The writers also favor the usage of Davis (11) in the interpretation of septation. That is, species that have mature spores predominantly 1-septate, yet may have a few spores 2-septate, or even 3-septate occasionally, especially late in the season, are retained in the genus *Ascochyta*. This includes *Stagonosporopsis* as a synonym, which is in agreement with Petrak (31).

The writers also favor including in the genus *Ascochyta,* species with slightly colored spores. This is in essential agreement with Saccardo's concept of the genus (**42**, v. 3) and also with that of Grove (**19**). Even in the type species, *A. pisi,* the senior writer has seen specimens on vetch with light brown spores. In correspondence in 1927, the late Dr. J. J. Davis attributed such slight coloring of otherwise hyaline spores to oxidation under conditions of intense sunlight. Other species also have slightly colored spores. This concept includes *Ascochytella* and *Ascochytula* as synonyms under *Ascochyta.* This is in agreement also with Petrak (**31**).

There seems to be justification for grouping into a separate section of the genus *Ascochyta,* the species with spores predominantly slightly colored, as Saccardo did in 1906 and again in 1913 (**42**, v. 18, 22) as follows:

"I. *Eu-Ascochyta.* Sporulae hyalinae.
II. *Ascochytella* Fl. Tassi (ut gen.). Sporulae dilute coloratae."

In the following pages, this plan will be followed. The plan using 3 sections, as suggested by Trotter (**59**), does not seem to be justified.

In the following pages, the species referred by some authors to the genus *Diplodina* will be included in *Ascochyta* as these genera essentially are synonyms, and the latter takes precedence. This too is in agreement with Petrak (**31**).

STUDIES CONDUCTED

Specimens listed under each species and available foreign collections were the basis for taxonomic study. Approximately the same methods were employed in this study as in that on *Septoria* (**48**), although virtually no host range studies have been made and pure culture isolations were made from only part of the collections.

The specimens collected before 1940 are filed in the Mycological Herbarium, Oregon State College, Corvallis, Ore., with duplicates, in part, in the Mycological Collections, Bureau of Plant Industry, Beltsville, Md. Specimens collected in the Northern Great Plains since 1939 are also filed at Beltsville and bear B.P.I. numbers in the 80,000 series. Material obtained since 1946 is filed in the

Department of Plant Pathology, State College of Washington, Pullman, Washington, in their collection series (C.S.) which, since 1947, has been changed to the Accession Series (A.S.). Slides prepared with the aid of the Works Progress Administration at Corvallis also are filed at Beltsville and some duplicates at Pullman.

The material studied totals 12 species or varieties of *Ascochyta* on 68 species of Gramineae. All but 8 of the 125 collections were obtained in twelve states as follows: North Dakota, 38; Oregon, 19; Washington, 18; Montana, 8; Idaho, 7; Utah, 6; Colorado, 5; Minnesota, California, and South Dakota, 4 each; and Wyoming and Iowa, 2 each. Besides the collections studied, Seymour (**44**) reported *A. graminicola* on *Calamagrostis canadensis* (Michx.) Beauv., *Poa annua* L., *Glyceria grandis* S. Wats., and *Agropyron smithii* Rydb. The files of MYCOLOGIA also contain a few references to *A. graminicola* on Gramineae but in general very little information is available on this group in the United States. Lefebvre and Howard Johnson (**24**) reported *A. graminicola* on *Agrostis alba* L., *A. canina* L., and *Poa pratensis* L. in the eastern United States. Preston (**35**) listed several additions from Oklahoma.

CLASSIFICATION

SECTION I. EU-ASCOCHYTA. Spores hyaline

1. ASCOCHYTA SORGHI Sacc.

A. graminicola Sacc. 1878 (**38**)
A. graminicola Sacc. sensu Davis (**11**)
A. graminicola var. *holci* Sacc. 1881 (**41**)
A. graminicola var. *leptospora* Trail 1887 (**57**)
A. graminicola var. *hispanica* Gonz. Frag. 1919 (**17**)
A. graminicola var. *diedickeana* Baudyš and Picb. 1926 (**4**)
A. elymi Tehon and Daniels 1927 (**56**)

Ascochyta sorghi is an obscure, late-winter parasite on the leaves of a number of grasses in Oregon and Washington. In the Rocky Mountains and Great Plains it develops in late summer, although at Mandan, N. Dak., it has been found as early as mid-May. It is particularly common in the Columbia River Gorge in Oregon and Washington, particularly on the shaded Oregon side of the river. Affected plants usually show a few red-bordered brown or fawn-colored lesions, mainly on the lower leaves or on the tips of foliage. Most of the material available is scanty. Sometimes, as on *Hierochloe odorata* (L.) Beauv., the fungus is actively parasitic.

DESCRIPTION

Pycnidia. Pycnidia are subglobose, erumpent, ostiolate, 90–140 μ in diameter, golden brown with darker oxidized cells adjacent to the ostiole. Material on *Arrhenatherum elatius* (L.) Presl. from near Corvallis, Ore., showed thin walls in cross section but the details of the pycnophores were not determinable.

Pycnospores. Some collections have spores as small as 11–14 × 2.5–3.0 μ on *Elymus condensatus* Presl (FIG. 1, *A*) but the spores of others are somewhat larger, ranging from 13–20 × 2.7–4.0 μ. On *Hierochloe odorata* the spores are fusoid to ovate-fusoid or subcylindric, 13–19 × 2.7–4.0 μ (FIG. 1, *B*); those on *Festuca rubra* L. are subcylindric, 15–20 × 3.2–4.0 μ (FIG. 1, *C*); on *Poa gracillima* Vasey they are subcylindric, 17–21 × 3.2–4.1 μ [cfr. Picbauer (**33**); Hennings (**21**, extr. 13, 14)]; on *Arrhenatherum elatius* from Oregon they are subcylindric, 11–18 × 1.6–3.1 μ (FIG. 1, *D*), whereas the type of *A. graminicola* was described by Saccardo (**38**) on this host as having ovate-fusoid, hyaline spores 10–12 × 4 μ. Material on *Stipa viridula* Trin. from near Webster, S. Dak., has spores 11–13 × 3.0–3.5 μ, but they were faintly chlorinous (FIG. 1, *E*). Spores in black pycnidia on *Avena sativa* L. from Tifton, Ga., also are cylindrical, 11–16 × 2.4–3.5 μ (FIG. 1, *F*), and material on *Poa pratensis* from Fargo, N. Dak. (FIG. 1, *G*), and on *Festuca rubra* from Mandan, N. Dak., had spores 12–16 × 3–4 μ (FIG. 1, *H*). A yellow spored collection on *Poa pratensis* from Jackson, Wyo., may be different.

DISCUSSION

Ascochyta graminicola Sacc., originally described on *Arrhenatherum avenaceum* Beauv. (*A. elatius*) and its various varieties, have been used as a convenient place to assign *Ascochyta*-like fungi on grasses. Collections in herbaria that are labeled *A. graminicola* include a few that are truly *Ascochyta*, but most of them are *Darluca filum* (Biv.) Cast., immature *Hendersonia, Stagonospora,* or *Septoria. Darluca filum* (Biv.) Cast, can be readily distinguished under relatively low magnification by the large, mammiform, frequently coalesced pycnidia growing in remnants of rust pustules. The spores are uniformly ovate, not cylindrical, broadest at the septum and, in some of the spores, the appendages can be vaguely seen. These deciduous appendages, however, often escape notice.

Davis (11) detailed the popular concept of *A. graminicola* as having subcylindric, fusoid to ovate-fusoid spores, 10–20 × 2.5–4.0 μ, borne in distinctly parenchymatous pycnidia with fuliginous walls. Davis' concept of the species and that of most workers would include var. *holci* Sacc. (41, p. 350) as well as *A. graminicola* var. *diedickeana* Baudyš and Picb., *A. elymi* Tehon and Daniels, and *A. sorghi* Sacc. It appears practical, therefore, to accept Davis' widely recognized emended description of *A. graminicola,* were it not necessary to refer this group to the earlier described *A. sorghi* Sacc., which was described as having spores 14 × 3 μ (37, p. 302).

Ascochyta graminicola var. *holci* Sacc. was described on *Holcus lanatus* L. from France as having spores 16–18 × 3–3.5 μ. Thus, as to spore size, it is in the upper ranges of *A. graminicola* but very close to *A. sorghi.* Much of the western material falls in this size range and, therefore, it is referred to *A. sorghi* (*A. graminicola*).

Ascochyta graminicola var. *diedickeana* Baudyš and Picb. was described (4) on *Bromus tectorum* L. from Bohemia as having spores 11–17 × 2.5–3.1 μ, thus easily within the range of *A. sorghi* (*A. graminicola*) to which it is referred. This fungus is definitely an active parasite and is rather common on *Bromus carinatus* Hook. and Arn. during early and mid-spring in western and intermountain Oregon. It produces brown-bordered lesions with paler centers and in severe cases a dark brown or nearly black general scalding of affected parts of the plant. Such material has been collected also on *Bromus inermis* Leyss. near Laporte, Minn. The pycnidia in a winter collection on *B. carinatus* at Hood River, Ore., are thin-walled, golden, ostiolate, 120–145 μ in diameter and contain subcylindrical spores (FIG. 1, *I*). The collection on the same host from Whitson, Ore., has slightly coarser, more fusoid spores, 15–20 × 3–4 μ (FIG. 1, *J*), and another collection on *B. inermis* from Minnesota has smaller spores (FIG. 1, *K*), which is clearly referable to *A. sorghi* (*A. graminicola*), and the resemblance to the Oregon material on *B. carinatus* is strong. *A. graminicola* was reported also on *B. cavanillesii* Willk. and *Cynosurus aureus* L. from Spain by Unamuno (61).

The variety *diedickeana* is not recognized as distinct because it differs from *A. graminicola* proper only in its strongly pathogenic nature, in the symptoms it produces, in its sometimes slightly narrower, straighter spores, and sometimes in its larger pycnidia, all of which characters are only relative. In passing, it is noted that symptoms induced by *Stagonospora bromi* Sm. and Ramsb. as

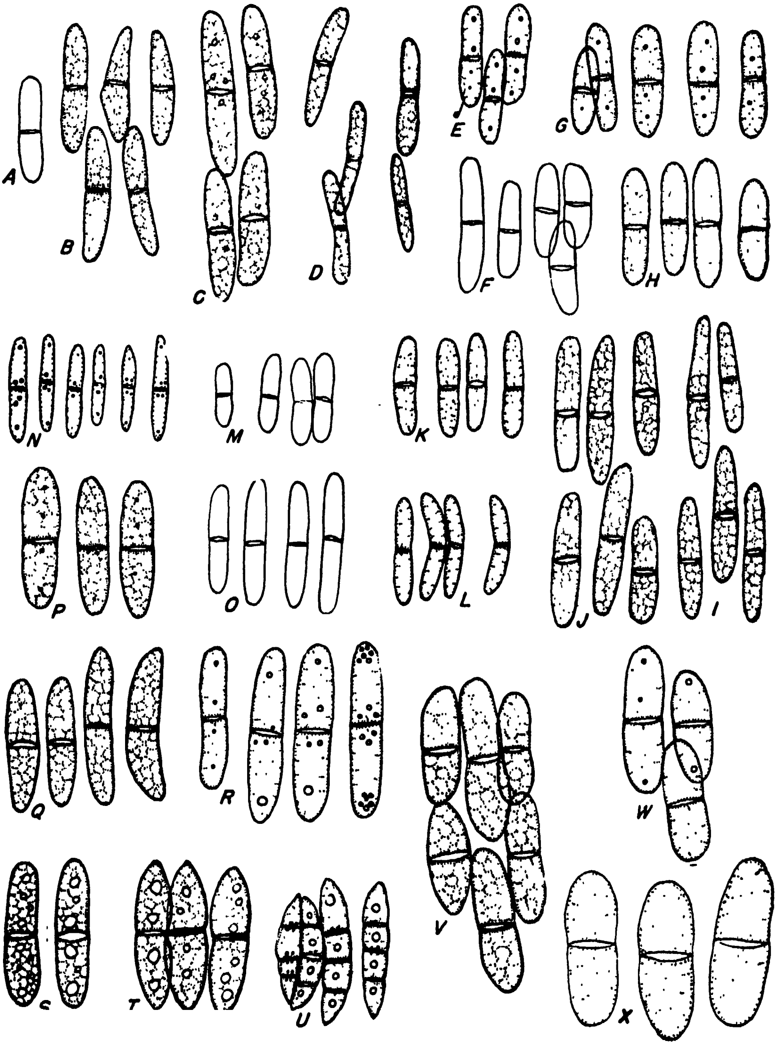

FIG. 1. Spores of *Ascochyta*.

shown by a number of collections of this fungus on *B. inermis* made by John Hardison at Ann Arbor, Mich. and by Sprague in North Dakota are similar to those caused by *A. sorghi* (*A. graminicola*) on this host.

Ascochyta sorghi (*A. graminicola*) was isolated from decayed basal leaf sheaths of *Bromus inermis* from McCanna, N. Dak. (47). The senior writer isolated over 30,000 pure cultures of fungi from the roots and basal portions of Gramineae in the Northern Great Plains during a six year period without encountering this fungus at any other time. The growth on potato dextrose agar was firm, cottony, gray and fuliginous with pycnidia imbedded in the matrix. The spores were hyaline, cylindrical to fusoid-cylindrical, 12–16 × 2.5–3.5 μ. Broadfoot (7) reported *A. graminicola* as occurring occasionally on the roots of cereals in Canada.

Ascochyta elymi Tehon and Daniels was described (56) on *Elymus virginicus* L. from Illinois as characterized chiefly by its thin-walled, pale pycnidia. The spores were described as 10–14 × 2–3 μ. The writers have not seen comparable material except on seedlings of *Elymus canadensis* L. growing on the banks of the Cannonball River, near Solen, N. Dak. We have, however, seen collections from several scattered points in the Rocky Mountains, also from Eugene, Ore., and from several places in the Northern Great Plains, which have spores 11–19 × 1.5–2.2 μ (FIG. 1, *L*) but which have pycnidia with walls somewhat browner and thicker than described for *A. elymi*. The one from Eugene, Ore., produced, in pure culture, a mucose, flesh-colored growth with aberrant, 1-septate spores, while material on *Agropyron desertorum* from Mandan produced a cottony, mottled, tawny and white growth. The Eugene, Ore., material is closer to *Septoria elymi* Ell. and Ev. (48) while the material on *A. desertorum* (Fisch.) Schult. cannot be assigned to that species, but may belong with the *A. sorghi* group.

Material of an *Ascochyta* leaf spot on *Sorghum vulgare* var. *sudanense* (Piper) Hitchc., collected by C. R. Ball in 1912 at Brookings, S. Dak., appears to be typical *A. sorghi* Sacc. (FIG. 1, *M*). In size and shape, the small, cylindrical spores, 11–12.5 × 3.2–3.9 μ, are very similar to *A. graminicola* on *Elymus condensatus* collected by G. Hamilton Martin at Pullman, Wash., in 1916 (FIG. 1, *A*). They are also similar to the smaller spores of the material of *A. graminicola* on *Avena sativa* from Tifton, Ga., collected by C. L. Shear (FIG. 1, *F*) and therefore all are assigned to *A. sorghi* (*A. graminicola*).

In passing it should be added that *A. graminicola* var. *hispanica* Gonz. Frag. (**17**, p. 117) on *Holcus lanatus L.*, which has ovate-fusoid spores, 9–11 × 2–2.5 μ, also is assignable to *A. sorghi,* while *A. festucae-erectae* P. Henn. (**21**, extr. 14) has still smaller spores, 6–9 × 2.5–3.0 μ, and may be distinct. The fungus reported by Gonzalez Fragoso (**18**) on *Hordeum* sp. from near Guadarrama, Spain, as *A. graminicola,* with spores 7–9 × 3 μ, also may possibly be distinct. Another collection by Gonzalez Fragoso on *Hordeum murinum* L. from Seville, Spain, reported by Saccardo (**43**, p. 285) as *A. graminicola,* with spores 12–16 × 3–4 μ, undoubtedly is *A. sorghi.* A confusion of immature stages of *Hendersonia* or *Stagonospora* with *A. graminicola* is frequent and almost unavoidable without detailed culturing in many instances. For example, the senior writer has collected material on *Agropyron smithii* near Clyde, N. Dak., showing what looked like three fungi in close association. Pure culture showed that they were all stages of *Hendersonia crastophila* Sacc.

Bisby *et al.* (**5**) reported *A. graminicola* on *Agropyron smithii* not far north of Clyde in Manitoba with spores 15–20 × 3–4 μ, and in suggested varieties, up to 30–40 μ long.

Excluded names:

A. graminicola var. *coeruleae* Br. and Har. = *Darluca filum* (Biv.) Cast.

A. graminicola var. *ciliolata* Sacc. = *D. filum.*

Specimens examined: [3] On *Agropyron desertorum* (Fisch.) Schult. (B.P.I. 81,022), Mandan, N. Dak., July 5, 1944; *A. spicatum* (Pursh) Scribn. and Sm. (B.P.I. 81,172), Belgrade, Mont., July 30, 1945; *A. smithii* Rydb. (A.S. 17,024), Roosevelt N. For., Colo. (15–20 × 2–3 μ [4]), Aug. 9, 1948; *Agrostis alba* L. (A.S. 17,006), Clearwater River, Idaho Co., Ida. (14–17 × 2.8–3.5 μ),

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[3] Specimens listed include only unreported or previously undetermined material; standard exsiccati and types are not repeated here.

[4] Measurements of pycnospores obtained from the collection.

Aug. 1, 1948; *A. scabra* Willd., Priest Lake, Ida.; *Arrhenatherum elatius* (L.) Presl (O.S.C. 21), Corvallis, Oregon (11–18 × 1.6–3 μ), Dec. 15, 1937; *Avena sativa* L., Tifton, Ga. (12–16 × 2.3–3.4 μ), April 11, 1931 (C. L. Shear); *Beckmannia syzigachne* (Steud.) Fern. (B.P.I. 80,620), Lisbon, N. Dak. (11–17 × 3.4–4.2 μ), 1939; *Bromus carinatus* Hook. and Arn., Whitson, Yamhill Co., Ore., June 17, 1939 (O.S.C. 689) (15–20 × 3–4 μ); Hood River, Ore., Feb. 26, 1935 (O.S.C. 10,200) (13–19 × 2.6–3.5 μ); (A.S. 20,042), Pullman, Wash., June 8, 1943; and (C.S. 3,619), Logan, Utah, June 6, 1947; *Bromus inermis* Leyss. (B.P.I. 80,270), McCanna, N. Dak., May 22, 1940; (B.P.I. 80,166), La Porte, Minn. (11–13 × 1.9–2.6 μ), June 4, 1941; Lexington, Ky. (13–16 × 2.5–3.8 μ), Oct. 1942; and (B.P.I. 80,621), Mandan, N. Dak., April 3, 1942; *B. tectorum* L. (B.P.I. 81,047), Detroit Lake, Minn., June 18, 1944; *Elymus canadensis* L., 25 miles west of Billings, Mont. (11–17 × 1.5–2.0 μ), July 19, 1941 (B.P.I. 80,275) (B.P.I. 80,223), Cannonball River near Solen, N. Dak., April 17, 1941, and (B.P.I. 81,183), Kingdom City, Mo. (13–16 × 3.2–3.9 μ), March 26, 1946; *E. condensatus* Presl, J. H. Martin 794, Pullman, Wash., Feb. 1916 (11–13.5 × 2.0–2.7 μ); *Festuca rubra* L., Newport, Ore., Dec. 18, 1937 (15–20 × 3–4 μ) (O.S.C. 35); Mandan, N. Dak. (12–16 × 3.9–4.2 μ), Sept. 9, 1940; Mandan, N. Dak., Sept. 14, 1940 (B.P.I. 80,034); and Garibaldi, Ore., June 23, 1937 (O.S.C. 8,377) (15.5–20 × 3.2–4.0 μ); *Hierochloë odorata* (L.) Beauv. (B.P.I. 80,271), Mandan, N. Dak. (13–19 × 2.7–4.0 μ), May 15, 1940; Mandan, N. Dak. (14–18 × 3.4–3.8 μ), May 30, 1916; and (B.P.I. 80,322), Rapid City, S. Dak., May, 1941; *Hordeum murinum* L., Culdesac Grade, Ida. (C.S. 3,612), June 4, 1947; *H. nodosum* L., Hunter, Alaska (15 × 2.4–3.0 μ); *H. jubatum* L. (B.P.I. 81,187), Panora, Iowa; *H. jubatum* × *Agropyron trachycaulum* (Lk.) Malte (*Elymus macounii* Vasey) (B.P.I. 81,046), north of Wilton, N. Dak., Aug. 3, 1944; and Townsend, Mont., Aug. 21, 1941; *Melica bulbosa* Geyer (A.S. 20,059), Shoshone Natl. For., Wyo. (13–18 × 2.3–2.8 μ); *Panicum capillare* L. (C.S. 3,316), Madison, Wisc., Oct. 1946; *Poa bulbosa* L. (C.S. 3,446), Pullman, Wash., April 4, 1947; *P. gracillima* Vasey (O.S.C. 10,280), Horse Tail Falls, Ore. (17–20 × 2–4.10 μ), May 14, 1935; (O.S.C. 10,939), Hood River, Ore. (16–20 × 3.2–4.0 μ), March 10, 1937; and Multnomah Falls, Ore. (16–19 × 3–4 μ), May 15, 1935; *P. pratensis* L. (B.P.I. 20,273), Fargo, N. Dak. (12–15.5 × 3–4 μ), Sept. 15, 1940; Jackson, Wyo. (12.8–14 × 3–3.8 μ), Sept. 15, 1940; (B.P.I. 81,185), Panora, Iowa, Mar. 27, 1946; *P. secunda* Presl, Belfield, N. Dak., June 19, 1945, and (O.S.C. 8,087), Maryhill Grade, Wash., Feb. 26, 1935; *Sclerochloa dura* (L.) Beauv. (C.S. 3,622), Culdesac Grade, Ida., June 4, 1947; *Sitanion hystrix* (Nutt.) J. G. Sm., Mt. Hood, Ore., July 4, 1936; *S. jubatum* J. G. Sm. (C.S. 17,162), Withrow, Wash. (14–17 × 2.8[illegible] μ), June 22, 1948; *Sorghum vulgare* var. *sudanense* (Piper) Hitchc., Brookings, S. Dak. (11–12.5 × 3.2–3.9 μ), July 29, 1912 (C. R. Ball); *Stipa occidentalis* Thurb. (C.S. 3,540), Malheur Natl. For., Ore., June 25, 1947; *Stipa viridula* Trin. (B.P.I. 80,277), Webster, S. Dak. (11–13 × 3.0–3.5 μ), June 6, 1941; *Trisetum canescens* Buckl. (A.S. 17,002), Idaho Co., Ida. (14–17 × 2.8–3.5 μ), Aug. 1, 1948; and (A.S. 20,144), Idaho Co., Ida. (11–17 × 2.6–3.5 μ), Aug. 1, 1948.

2. ASCOCHYTA PHLEINA Sprague

Ascochyta phleina, which has been described elsewhere (**50**), causes tawny spots on the leaves of *Phleum pratense* L. collected near Lake George, Niawa, Minn. (B.P.I. 80,920). The spores are cylindrical, tapering slightly toward the rounded ends, not constricted at septa, 11–16 × 1.5–2.2 μ (FIG. 1, *N*). This species has a stouter pycnidium than that of *A. elymi* and the spores are narrower than in the type of the latter. It appears to be moderately parasitic.

3. ASCOCHYTA DESMAZIERI Cav.

Ascochyta desmazieri also has been discussed elsewhere (**48, 49**). It is an important parasite of *Lolium spp.* in the Willamette Valley, Oregon, and occurs also in western Washington. W. B. Cooke also sent us material from the vicinity of San Francisco, Calif. It occurs on both *L. perenne* L. and *L. multiflorum* Lam. in these areas, but is most prevalent on the latter host. The typical, truly cylindrical spores (FIG. 1, *O*) and the well defined chocolate brown spots distinguish this species.

Specimens examined: On *Lolium multiflorum* Lam. (O.S.C. 10,217), Corvallis, Ore. (13–15 × 2.4–2.6 μ), Jan. 10, 1935; (O.S.C. 10,235), Corvallis, Ore. (12–18 × 2.4–3.1 μ), Jan. 8, 1935; (O.S.C. 10,122), Corvallis, Ore. (12–17 × 2.4–2.9 μ), Jan. 20, 1934; (O.S.C. 733), Corvallis, Ore. (13–18 × 2.3–3.0 μ), Dec. 8, 1939; and Puyallup, Wash. (A.S. 3,931), June 7, 1948; *L. perenne* L. (O.S.C. 10,219), Corvallis, Ore., Dec. 15, 1934; (Cooke 16,503), near San Francisco, Calif. (15–20 × 2.3–2.8 μ), 1941.

4. **Ascochyta brachypodii** (Sydow) comb. nov.

Ascochyta graminicola var. *brachypodii* Trail, 1887 (**57**)
Diplodina brachypodii Sydow, 1916 (**53**)

This fungus was described by Trail (**57**) in 1887 as *Ascochyta graminicola* var. *brachypodii* on *Brachypodium sylvaticum* (Huds.) Beauv. from Scotland as having slightly fusoid spores, with blunt ends, 15–17 × 5 μ. Sydow (**53**), in 1916, described apparently the same fungus as *Diplodina brachypodii* on *B. sylvaticum* from Germany as having cylindric-fusoid, hyaline, obtuse ended spores 16–22 × 5–6 μ. It is evident, therefore, that these two names apply to the same fungus, which differs distinctly from *Ascochyta sorghi* (*A. graminicola*).

Much of our western material is represented by small collections with scattered pycnidia. Spores vary somewhat in the several collections (FIGS. 1, *P, Q, R, S*). Most of them indicate weak parasitism of the associated fungus, but one collection made on *Stipa viridula* from Sprague Siding, Minn., appears to be actively parasitic (B.P.I. 80, 567).

Ascochyta graminicola var. *aciliolata* Alm. and Camara (3) on *Lolium italicum* L., *L. perenne* L., and *Festuca pratensis* A. Br. from Portugal has oblong, cymbiform spores 15–18 × 4.5–6.0 μ. Gonzalez Fragoso (**16**, p. 699), who probably had access to the type, listed it from Spain on *Avena filifolia* Lag. var. *scabra* Wk. with spores 21 × 7 μ. On the basis of spore size, var. *aciliolata* apparently is not assignable to *A. brachypodii.*

The writers have studied two collections, one on *Andropogon gerardi* Vitman (A. *furcatus* Muhl.) (B.P.I. 80,283) and the other on *Sorghastrum nutans* (L.) Nash (B.P.I. 80,878), that have broadly spindle-form spores (FIG. 1, *T*), which appear somewhat different from *A. brachypodii.* Material from *Sorghastrum nutans,* however, which had the characteristic spindle-form spores on the host, eventually produced cylindrical pycnospores 15–21 × 4.2–5 μ that were identical with those of *A. brachypodii* when grown in culture on sterilized culms of crested wheatgrass. For this reason the writers hesitate to name the material on *A. gerardi* and *S. nutans* as new until further collections are available. Therefore, even though obviously somewhat different, the fungus on these two grasses is tentatively included under *Ascochyta brachypodii,* but the following special description is given:

Spots oblong, striate or diffuse, sordid isabelline to ashy or fawn colored in center, emarginate or sometimes bordered with rufous to vinaceous pigmented areas, pycnidia subnumerous, more or less gregarious, creosote brown, finally parenchymatous, sunken, later erumpent, globose to sub-mammiform, ostiole small, slightly protruding, ringed by darker cells, 95–144 μ; pycnospores (in nature) spindleform, straight, ends pointed, slightly constricted at the 1 (rarely 2) septum, contents 2–several guttulate (1–2 μ diameter), hyaline or faintly tinted, 17–21 × 4.2–5.0 μ. On living leaves of *Andropogon gerardi* at Mandan, N. Dak., June 10, 1941, and on *Sorghastrum nutans,* Mandan, Sept. 14, 1943. This fungus was first collected during a humid period at Mandan and appears to be moderately parasitic in the somewhat fragmentary material thus far available. The spores on *A. gerardi* (FIG. 1, *T*) are definitely larger than those of *Ascochyta ischaemi* Sacc. (10 × 4.5 μ), which occurs on *Andropogon ischaemum* L. in Italy.

In this connection, it may be added that some time ago, we received from W. W. Ray a collection of a species of *Stagonospora* similar to *St. ischaemi* Sacc. This collection, which was made by K. Starr Chester on *Andropogon gerardi* near Stillwater, Okla., had 3-septate, fusiform, chlorinous spores 16–18 × 2.7–4.2 μ (FIG. 1, *U*) borne in well-defined tawny spots. Preston (**35**) has reported *St. ischaemi* on *A. furcatus* Muhl. (= *A. gerardi*) from Oklahoma. This appears to be the most satisfactory place to file this species at present. Since the North Dakota *Ascochyta* collection (FIG. 1, *T*) has larger spores than *St. ischaemi* and is only 1-septate, it is considered distinct from it.

Specimens examined: On *Andropogon gerardi* Vitman (B.P.I. 80,283), Mandan, N. Dak. (17–21 × 4.2–5.0 μ), June 10, 1941; *Avena sativa* L., Dickinson, N. Dak., 1946; *Chloris virgata* Swartz (B.P.I. 80,867), Mandan, N. Dak. (16–20 × 4.4–4.9 μ), Aug. 27, 1943; *Lolium perenne* L. (O.S.C. 10,239), near Troutdale, Ore. (15–18 × 3.5–4.3 μ), Feb. 26, 1935; *Melica virgata* Turcz., Mandan, N. Dak., July 10, 1945; *Phalaris arundinacea* L. (B.P.I. 80,282), Mandan, N. Dak. (16–20 × 4.3–4.7 μ), Oct. 1, 1941; *Pleuropogon refractus* (A. Gray) Benth. (O.S.C. 197), Bergsvik Creek, Ore. (17–20 × 4.5–4.7 μ), May 16, 1938; *Poa cusickii* Vasey (C.S. 3,901), Cow Creek, Ore., April 6, 1948; *Sorghastrum nutans* (L.) Nash (B.P.I. 80,878), Mandan, N. Dak. (15–21 × 4.2–5.0 μ), Sept. 14, 1943; *Stipa comata* Trin. and Rupr. (B.P.I. 80,272), Hebgen Lake, Mont. (18–23 × 4.0–4.3 μ), Aug. 17, 1941; *S. viridula* Trin. (B.P.I. 80,567), Sprague Siding, Minn. (15–18 × 4.3–5.4 μ), June 17, 1939.

5. **Ascochyta graminea** (Sacc.) comb. nov.

Diplodia graminea Sacc. 1881 (**40**)
Diplodina graminea Sacc. 1884 (**42**)
Ascochyta boutelouae Fairman 1918 (**15**)
Ascochyta cynodontis Unam. 1929 (**60**)

Diplodina graminea Sacc. was described originally by Saccardo (**40**, p. 267) as *Diplodia graminea* on *Cynodon dactylon* (L.) Pers. from Padua, Italy, and later (**42**, v. 3) transferred to the genus *Diplodina*. It was described as having oblong spores 15–16 × 5–7 μ borne in minute pycnidia. Diehl (**14**) reported this species on this grass from South Carolina in 1935, with spores 11–13 ×

4–5 μ. In May 1939, the senior writer collected material on the same grass near Davis, Calif., which appears to be the same species as that reported by Diehl. It contained subsuperficial pycnidia scattered among *Helminthosporium cynodontis* Marig. The pycnidia were dark brown, tardily ostiolate, long-ellipsoidal, 100–160 × 50–90 μ. The pycnidial wall was composed of large polygonal cells. The spores are distinct from those of the *Ascochyta sorghi* (*A. graminicola*) group. They are oblong-ellipsoid, 1-septate, clear-hyaline but with a hint of smokiness in mass, 11–18 × 3.9–5.0 μ (FIG. 1, *V*).

Ascochyta cynodontis Unam. (**60**), which appears to be the same fungus, has been described from Spain on *Cynodon dactylon.* Unamuno reported immersed, globose-depressed, brown, fuliginous pycnidia 88.5–125 × 53.5–67.8 μ with hyaline, ellipsoid, 1-septate spores, 12–18 × 3.5–4 μ.

What appears to be the same species or a form of it occurs on *Bouteloua gracilis* (H. B. K.) Lag. in the western United States in the Rocky Mountains and eastward. This fungus, *Ascochyta boutelouae* Fairman (**15**), was described from the mountains of New Mexico with hyaline or greenish hyaline spores 17–20 × 6–7 μ. Most of the plains material in North Dakota, however, has spores 15–18 × 4.5–6 μ and like the *Cynodon* material, the spores have a hint of smokiness in mass, as well as having the same oblong-ellipsoid shape (FIG. 1, *V* and *W*).

The pycnidia of *A. boutelouae* were described as being either small (55 μ) or often oblong (90–150 μ). They are, therefore, similar to the material on *Cynodon* from Davis, Calif. The material collected on *Bouteloua* in North Dakota has similar, elongated pycnidia. The writers feel, therefore, that only one species is involved, notwithstanding the fact that the fungus on *Bouteloua* tends to have slightly broader spores.

The *Diplodina*-like, or carbonaceous condition of the pycnidia is attributable to the siliceous nature of the substratum, particularly on *Cynodon.* The writers refer this species to the genus *Ascochyta.* This species apparently is weakly, if at all, parasitic.

Specimens examined: On *Bouteloua gracilis* (H. B. K.) Lag. (B.P.I. 80,578), Mandan, N. Dak. (15–18 × 4.5–6.0 μ), June 20, 1941 (B.P.I. 80,221),

Pingree, N. Dak. (15–17 × 4.5–6.0 μ), July 30, 1941; *Cynodon dactylon* (L.) Pers. (O.S.C. 10,910A), Davis Jc., Calif. (11–18 × 3.9–5.0 μ), May 17, 1939.

6. ASCOCHYTA SORGHINA Sacc.

Ascochyta sorghina was described by Saccardo (39), in 1878, on leaves of *Sorghum vulgare* L. from Italy as follows:

> Maculis longitudinaliter oblongis arescendo fuscis, sanguineomarginatis; peritheciis dense gregariis, globoso-depressis, papillulatis pro genre majusculis; spermatiis oblongo-ellipsoideis, 20 × 8, 1-septatis, leniter constrictis, minute pluriguttulatis, hyalinis.
>
> ---------------
>
> *Obs.* Ab. *A. zeina* peritheciis majoribus, spermatiis pluriguttulatis etc. recedere videtur.

This description was repeated *verbatim* by Saccardo (42, v. 3) in 1884. The large, blunt pycnospores (FIG. 1, *X*) are strikingly different from those of *A. sorghi,* as are also the pycnidia. The pycnidia of *A. sorghina* are so large that they protrude so conspicuously that they make the lesions feel rough to the touch. Because of this, the name "Rough Spot" has been applied by Leukel *et al.* (25) to the disease caused by this fungus. *A. sorghina* attacks not only various varieties of sorghum but also Sudan grass (*Sorghum vulgare sudanense*) and Johnson grass (*S. halepense* (L.) Pers.) in the southeastern United States.

ASCOCHYTA SPARTINAE Trel. (58). This species was transferred to the genus *Septoria* as *S. spartinae* (Trel.) Sprague (48) because the hyaline spores were 1–3-septate and too long in proportion to their width (35 × 3 μ) for the genus *Ascochyta.*

SECTION II. ASCOCHYTELLA Tassi. Spores slightly colored

7. **Ascochyta subalpina** sp. nov.

Maculis nullis, pycnidiis depresso-globosis usque mammiformibus, nigro-brunneis, ostiolatis, erumpentibus 90–210 μ diam.; pycnosporulis cylindraceis, utrinque rotundatis, hyalinis v. chlorinis, guttulatis, 1-septatis, 11–18 × 1.9–3.0 μ.

Hab. in foliis emortuis *Deschampsiae elongatae,* Mt. Rainier National Park, Wash.; *Distichlidis strictae,* Marion, Montana (B.P.I. 80,045, typus); *Oryzopsidis asperifoliae,* Spearfish Creek, S. Dak.

Spots none, pycnidia globose-depressed to mammiform, black to brown, strongly pigmented with well developed ostioles and closely

knit walls, very strongly erumpent, scattered to loosely grouped, 90–210 μ; pycnospores cylindrical with straight sides and blunt, rounded ends not constricted at the single, central septum, context at first hyaline with few or no oil droplets, later with small greenish to pale, yellow-brown droplets and one moderate sized oil droplet in each cell, very numerous, fairly uniform in size, mostly 12.5 × 2.2 μ, ranging from 11–18 × 1.9–3.0 μ.

This fungus was found on a herbarium specimen of *Distichlis stricta* (Torr.) Rydb., (N–9–105), filed in the Soil Conservation Service collections at the Northern Great Plains Field Station, Mandan, N. Dak. The specimen was collected by G. L. Weber, July 7, 1936, in the mountains near Marion, Montana. The type is a fragment (B.P.I. 80,045) taken from this herbarium specimen.

Ascochyta subalpina apparently is an overwintering saprophyte. On *Distichlis stricta*, it is associated with *Leptosphaeria* sp., *Mycosphaerella* sp. and with *Puccinia aristidae* Tracy. The fungus is characterized by its cylindrical, capsular spores (FIG. 2, *A*). These spores differ from those of *A. phleina*, which are hyaline and taper slightly toward the ends. The spores of *A. subalpina* are narrower than those of the *A. sorghi* (*A. graminicola*) group and in addition are tinted. The ends of the spores are blunt and do not tend to be sublanceolate as in the one-septate phase of *Septoria oudemansii*. *A. subalpina* is not to be confused with *Hendersonia culmicola* var. *minor* Sacc. because the juvenile form of this variety has narrowly fusoid or lanceolate, pale yellow, 1-septate spores.

Specimens examined: Deschampsia elongata (Hook.) Munro, Mt. Rainier National Park, Wash., Sept. 21, 1946 (15.5–18 × 1.9–2.4 μ); *Distichlis stricta* (Torr.) Rydb. (B.P.I. 80,045), Marion, Mont. (type), July 7, 1936 (11–13 × 2.0–2.4 μ); *Oryzopsis asperifolia* Michx. (B.P.I. 81,190), (Upper) Spearfish Creek, S. Dak., June 27, 1946 (12–16 × 2.0–3.0 μ).

8. ASCOCHYTA AGROPYRINA (Fairman) Trotter

Ascochyta agropyrina was described originally by Fairman (15) as *Ascochytula agropyrina* Fairman on *Agropyron bakeri* E. Nels. from the mountains of New Mexico. It has very large globose pycnidia, 250–320 μ in diameter, and oblong-cylindrical, flavid to greenish, finally light brown spores, 17–23 × 6 μ. Trotter (59) transferred the species to the genus *Ascochyta* as he did not recognize *Ascochytula* as a genus.

The specimens available on species of *Elymus* and *Agropyron* that we are assigning to *Ascochyta agropyrina* are variable. One collection on *E. virginicus* from Mandan, N. Dak., has brown, later tawny spots in which prominent black pycnidia occur, 130–165 μ in diameter. The spores in this collection are hyaline to greenish with relatively homogeneous contents, subcylindrical with one cell tending to be smaller and sharper pointed than the other, 19–21 × 5.5–6.2 μ (FIG. 2, *B*). The other collections, several from Mandan, N. Dak., and westward, show spores somewhat smaller than typical for the species, but most of them are summer spores, which usually average smaller than spores formed during cooler seasons of the year. The spores are yellow to pale brown, 14–19

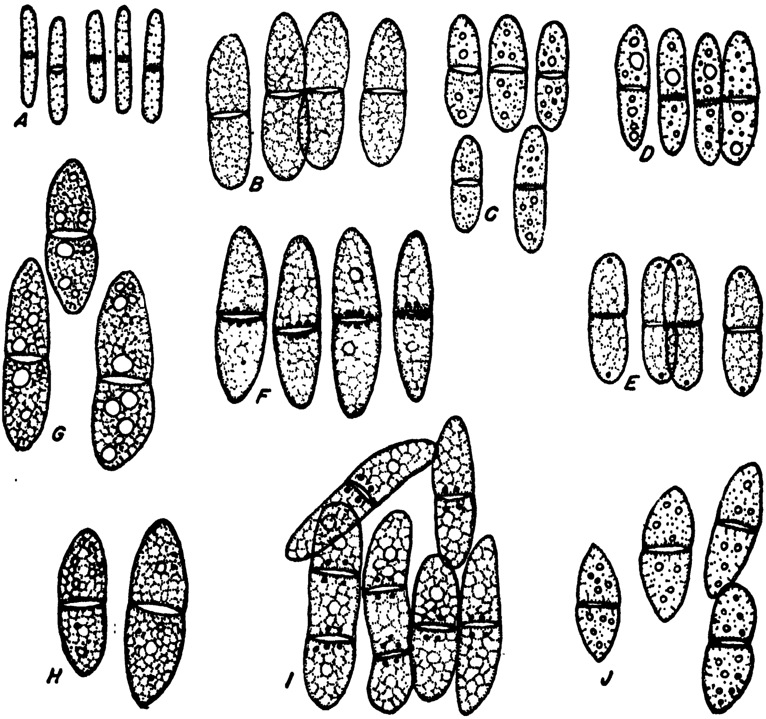

FIG. 2. Spores of *Ascochyta*.

× 4.5–6.2 μ (FIG. 2, *C, D*). Much of this material appears to be saprophytic or essentially so. A collection on *Agropyron subsecundum* (Link) Hitchc. from Pullman, Washington, however, is associated with fuscous spots with red vinaceous borders. The spores in this collection are very pale yellow, but of the stout, broadly cylindrical outline characteristic of *Ascochyta agropyrina* (FIG. 2, *E*).

Specimens examined: On *Agropyron caninum* (L.) Beauv. (B.P.I. 80,263), Mandan, N. Dak., June 13, 1941; and Mandan, N. Dak. (15–20 × 4.5–5.2 μ), July 1, 1941; *A. cristatum* (L.) Gaertn. (B.P.I. 80,265), Forsythe, Mont., Aug. 14, 1941; (B.P.I. 80,619), Mandan, N. Dak., July 7, 1941; and (C.S. 3,941), Pullman, Wash., June 14, 1948; *A. michnoi* Roshev., Mandan, N. Dak., July 1, 1941; *A. semicostatum* (Steud.) Nees, Pullman, Wash. (15–18 × 4.5–5.0 μ), July 15, 1941; and (B.P.I. 80,173), Mandan, N. Dak. (15.5–19 × 4.8–5.0 μ), June 13, 1941; *A. sibiricum* (Willd.) Beauv., Mandan, N. Dak. (13–21 × 4.4–5.6 μ), July, 1943; *A. smithii* Rydb., Forsythe, Mont. (15–18 × 5.0–6.2 μ), Aug. 14, 1941; *A. spicatum* (Pursh) Scribn. and Sm., Belgrade, Mont., Aug. 1948; *A. subsecundum* (Lk.) Hitchc. (B.P.I. 80,267), Mandan, N. Dak. (14–17.4 × 4.5–5.0 μ), June 13, 1941; and Pullman, Wash., 1941; *A. trachycaulum* (Lk.) Malte (B.P.I. 80,226), Lawton, N. Dak., July 29, 1941; (B.P.I. 81,119), Dundurn, Sask., Aug. 3, 1941; and (A.S. 20,129), Pass above Sheep Creek, Utah, Aug. 4, 1948; *A. trichophorum* (L.) Richt. (B.P.I. 80,266), Mandan, N. Dak. (17–19 × 4.7–5.4 μ), June 13, 1941; *Elymus canadensis* L. (B.P.I. 80,264), Mandan, N. Dak., June 13, 1941; *E. virginicus* L. (B.P.I. 80,261), Mandan, N. Dak. (19–21 × 5.5–6.2 μ), June 13, 1941.

9. ASCOCHYTA UTAHENSIS Sprague

Ascochyta utahensis, which has been described elsewhere (51), has very large, lightly tinted spores 22.6–29 × 6.6–10 μ. Unlike *A. agropyrina,* it occurs in well defined spots and apparently is actively parasitic. It occurs on *Agropyron inerme* (Scribn. and Sm.) Rydb. (type) in Utah and *A. trachycaulum* in Wyoming and Colorado. It was found in three locations in the mountains of Colorado.

10. ASCOCHYTA HORDEI Hara

Ascochyta hordei has been discussed previously (**51**). According to Ideta (**23**), it was described by Hara (**20**) in Japan apparently on wheat and barley. (The writers have not seen the original paper.) Ideta reports that the fungus causes round or irregular spots on the leaves of barley and rye, especially in March and April. He reports spherical to sub-spherical pycnidia 100–150 μ in diameter and 1-septate, hyaline or yellowish, oval, egg-shaped or cocoon-shaped spores with both ends rounded, 17–28 × 4–6 μ.

Certain collections in some of the Western states on *Bromus carinatus, Festuca elatior* L., *Holcus lanatus, Hordeum murinum,* and *H. vulgare* L. fit the above description fairly well and are, therefore, tentatively referred to this species. If the original description or the type specimen can be made available, possibly it may be found desirable to segregate some of the above material as a variety, as the spores, particularly on *Holcus lanatus* and *Hordeum vulgare,* are somewhat less obtuse at both ends and also somewhat more colored than those on the other hosts.

This species is apparently a winter parasite or saprophyte on winter barley (*Hordeum vulgare*) growing on the wind-swept high prairie region (**46**, FIG. 1, *A* and 2, *A*) in Klickitat County, Washington, adjacent to the Columbia River. The collection on *Holcus lanatus* was made several miles away on the floor of the valley below High Prairie, Washington.

Two collections on *Holcus lanatus* had broadly fusiform to ellipsoid spores 20–27 × 5–7 μ (FIG. 2, *F*), with small oil droplets adjacent to the septum. On *Hordeum vulgare,* the spores were slightly larger, 19–29 × 5–8.9 μ (FIG. 2, *G*). The pycnidia were subglobose 120–160 μ in diameter, clustered in gray to ill defined areas on dead leaves. This fungus apparently is slightly less rugged on *Holcus lanatus* than on *Hordeum vulgare,* but on both hosts is very distinct from *A. sorghi* on *H. lanatus,* which was reported by Gonzalez Fragoso (**17**, p. 117) on *H. lanatus* as *Ascochyta graminicola* var. *hispanica,* with spores 9–11 × 2–2.5 μ, as previously noted.

PURE CULTURE STUDIES

Isolations of *Ascochyta hordei* were readily obtained from barley leaves. After 15 days at 42° F., the colonies on potato dextrose agar were 7–10 mm. in diameter. From below, they were olive green with many black pycnidial initials scattered through the substratum. From above they were olive-gray, loose, and velvety with a heavier ring of growth near the margin. The mycelia, which consisted of rugged rectangular cells, were smoky and coarse, with hyphae 4–5.5 μ in diameter. Older transfers produced a moderately cottony, medium gray-brown colony with a darker substratum. Pycnidia were scattered through the mycelial mats but most of them formed at the edge of the colonies where the hyphae contacted the glass. Typical yellow-brown spores were produced in pure culture (FIG. 2, *H*). Pycnidia were produced sparingly or not at all one year after isolation. Lighter buff to isabelline tones of staling hyphae had developed on the gray and brown mycelia. The cultures were similar macroscopically to one of the isolations of *Hendersonia graminis* McAlp. isolated from wheat in Oregon and Washington.

Inoculations on six weeks old seedlings of Olympia barley, Winter Turf (Gray Winter) oats (*Avena sativa*), and Golden wheat (*Triticum aestivum* L.) with sporulating pure cultures of *Ascochyta hordei* and with barley leaves with pycnidia gave negative results under cool greenhouse conditions at Corvallis, Oregon. At most, the fungus apparently is a weak parasite on over-wintering plants. It is relatively common on barley in the High Prairie area of Washington in early April just after the snow has melted.

Specimens examined: On *Bromus carinatus,* Logan, Utah (C.S. 3,852) (17.8–21 × 5.3–7.1 μ); and near Otis Orchards, Washington (C.S. 19,981), March 22, 1947; *Festuca elatior* near Logan, Utah (C.S. 3,682 and 3,856) (16–22 × 4.8–6.2 μ), June 7, 1947; *Holcus lanatus* near Bingen, Wash., Feb. 26, 1935 (O.S.C. 8,081) (20–27 × 5–7 μ); and near Bingen, Wash., Feb. 26, 1935 (O.S.C. 8,089) (20–25 × 6–7 μ); *Hordeum murinum,* Riggins, Idaho (C.S. 3,677), June 4, 1947; *H. vulgare,* High Prairie, Wash., March 30, 1937 (O.S.C. 10,959 and B.P.I. 80,280) (20–27 × 5.0–7.7 μ); High Prairie, Wash., March 20, 1939 (O.S.C. 771) (19–29 × 5.2–8.7 μ); *Phleum phleoides* (L.) Karst., Pullman, Wash., May 1, 1948 (C.S. 3,923); *Stipa robusta* Scribn., Cedaredge, Colorado, August 6, 1948 (A.S. 17,087).

11. ASCOCHYTA AVENAE (Petrak) Sprague and A. G. Johnson

Ascochyta avenae (Petr.) Sprague and A. G. Johnson (*Ascochytella avenae* Petrak) has been briefly discussed previously (**51**). It has been collected twice in the far west, once in late winter (March 13, 1935) on red oats (*Avena byzantina* C. Koch) at Troutdale, Oregon (O.S.C. 10,243), and once in summer at Pullman, Wash., on wild oats (*Avena fatua* L.). When collected, the Oregon material had hyaline, 1- to 2-septate spores, 17–26 ×

6.2–7 μ, but a year later they had become slightly yellow tinted. An attempt was made to compare them with the type of *Ascochytella avenae* Petrak but the specimen available to the writers disclosed only telia of *Puccinia coronata* Corda. The Oregon material answers Petrak's (31) carefully detailed description. The Oregon specimen has prominent, dark pycnidia 100–140 μ, borne in pale, necrotic spots on the overwintering leaves. The spores (FIG. 2, *I*) were irregularly cylindrical to fusoid with rounded ends and coarse granular contents. The collection from Washington appears to be summer material of the same species. There was present also an associated *Mycosphaerella*-like fungus but it was too immature to identify.

Ascochyta avenae is related to *A. agropyrina* (Fairman) Trotter.

12. **Ascochyta missouriensis** sp. nov.

Maculis griseis, emarginatis saepe nullis; pycnidiis sparsis, nigris, erumpentibus, subpapillatis, 80–120 μ; pycnosporulis cymbiformibus, ad septum constrictulis, apicibus subacutis, 1-septatis, hyalinis, guttulis plus minusve conspicuis flavis tinctis, 15–19 × 6–8 μ.

Hab. in foliis dejectis *Panici virgati*, Wilton, N. Dak. (B.P.I. **80,279, typus**).

The species is characterized by thick, short, boat-shaped spores, which are slightly constricted at the septa and have rather sharp ends (FIG. 2, *J*). The inclusions are yellow, several to a cell, and comparatively prominent. Technically this species has hyaline spores but the yellow inclusions tend to give it a tinted appearance. It is therefore placed in section *Ascochytella* as the spores are coarse and the inclusions yellow. It seems, therefore, to belong with this group rather than with the strictly hyaline group, *Eu-Ascochyta.*

This species is probably of little economic importance. The senior writer has collected fragmentary material of this species several times on leaves of *Panicum virgatum* L. in the vicinity of Mandan, N. Dak. The type consists of a few selected leaves of authentic material plus a considerable quantity of undifferentiated material from the same collection. The fungus never appears to be common. This species has been collected also on *Panicum dichotomiflorum* Michx. at Ft. Totten, N. Dak.

Ascochyta missouriensis is named for the Missouri River near which it was found.

Specimens examined: On *Panicum virgatum* L. (B.P.I. 80,279), near Wilton, N. Dak., Sept. 15, 1941 (15–19 × 6–8 μ); Mandan, N. Dak., Sept. 20, 1944 (15–18 × 6.0–7.6 μ); and near Mandan, N. Dak., Sept. 6, 1945 (16–18 × 6–7 μ); *P. dichotomiflorum* Michx., Ft. Totten, N. Dak., Aug. 4, 1944.

SUMMARY

The genus *Ascochyta* is considered as including sphaeropsidaceous species with chiefly 2-celled, hyaline or slightly colored spores (Sections: I, *Eu-Ascochyta;* II, *Ascochytella*). This includes the following genera: *Ascochytella* Tassi, *Ascochytula* Died., *Stagonosporopsis* Died., and *Diplodina* West.

The following key will serve to segregate the species of *Ascochyta* on Gramineae that have been studied.

SECTION I. EU-ASCOCHYTA: Spores hyaline

- A. Spores chiefly less than 4.0 μ in diameter
  - B. Spores rather narrowly fusiform—ellipsoid
    - C. Spores chiefly 2.5 μ or more in diameter.....1. *A. sorghi*
    - CC. Spores chiefly less than 2.5 μ in diameter....2. *A. phleina*
  - BB. Spores narrowly cylindrical with blunt ends....3. *A. desmazieri*
- AA. Spores chiefly more than 4.0 μ in diameter
  - B. Spores chiefly less than 5.0 μ in diameter, rather broadly cylindrical with tapering ends........................4. *A. brachypodii*
  - BB. Spores oblong-ellipsoid, up to 5 μ or more in diameter, with blunt ends
    - C. Spores usually up to 6 μ in diameter......5. *A. graminea*
    - CC. Spores up to about 8 μ in diameter........6. *A. sorghina*

SECTION II. ASCOCHYTELLA *Tassi:*

Spores slightly colored or tinted by colored inclusions

- A. Spores narrowly cylindrical, with blunt ends, chiefly 3 μ or less in diameter..7. *A. subalpina*
- AA. Spores more than 4 μ in diameter
  - B. Spores broadly cylindrical with more or less blunt ends, scarcely at all constricted at septum
    - C. Spores tapering rather abruptly to blunt ends
      - D. Spores chiefly less than 6 μ in diameter.......... 8. *A. agropyrina*
      - DD. Spores chiefly more than 6 μ in diameter......... 9. *A. utahensis*

CC. Spores tapering gradually to rather blunt ends
  D. Spores broadly fusiform to ellipsoid, seldom 2-septate............................10. *A. hordei*
  DD. Spores irregularly cylindrical to ellipsoid, some 2-septate...........................11. *A. avenae*
BB. Spores broadly ellipsoid with rather sharply pointed ends, slightly constricted at septum.....................12. *A. missouriensis*

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EXPLANATION OF FIGURES

FIG. 1.—Pycnospores of species of *Ascochyta* on Gramineae in the section Eu-Ascochyta: *A–M, Ascochyta sorghi* Sacc.: *A,* on *Elymus condensatus,* Pullman, Wash., 1916 (G. H. Martin 794); *B,* on *Hierchloë odorata,* Mandan, N. Dak., May 15, 1940; *C,* on *Festuca rubra,* Garibaldi, Oregon, June 23, 1937 (O.S.C. 8,377); *D,* on *Arrhenatherum elatius,* Corvallis, Oregon, Dec. 15, 1937 (O.S.C. 21); *E,* on *Stipa viridula,* Webster, S. Dak., June 6, 1941; *F,* on *Avena sativa,* Tifton, Ga., April 11, 1931; *G,* on *Poa pratensis,* Fargo, N. Dak., Sept. 15, 1940; *H,* on *Festuca rubra,* Mandan, N. Dak., Sept., 1940; *I,* on *Bromus carinatus,* Hood River, Oregon, Feb. 26, 1935 (O.S.C. 10,200); *J,* on *B. carinatus,* Whitson, Oregon, June 17, 1939 (O.S.C. 689); *K,* on *Bromus inermis,* Laporte, Minn., June 4, 1941 (B.P.I. 80,166); *L,* on *Elymus canadensis,* west of Billings, Mont., July 19, 1941; *M,* on *Sorghum vulgare* var. *sudanense,* Brookings, S. Dak., July 29, 1912; *N, A. phleina* Sprague on *Phleum pratense,* near Lake George, Niawa, Minn., June 4, 1941 (B.P.I. 80,920); *O, A. desmazieri* Cav. on *Lolium multiflorum,* Corvallis, Oregon; *P, A. brachypodii* (Sydow) Sprague and A. G. Johnson on *Phalaris arundinacea,* Mandan, N. Dak., Oct. 1, 1941 (B.P.I. 80,282); *Q, A. brachypodii* on *Lolium perenne,* Troutdale, Oregon, Feb. 26, 1935 (O.S.C. 10,239); *R, A. brachypodii* on *Stipa comata,* Hebgen Lake, Mont., Aug. 17, 1941 (B.P.I. 80,272); *S, A. brachypodii* on *Pleuropogon refractus,* Bergsvik Creek, Oregon, May 16, 1938 (O.S.C. 197); *T, A. brachypodii* on *Andropogon gerardi,* Mandan, N. Dak., June 10, 1941 (B.P.I. 80,283); *U, Stagonospora ischaemi* Sacc. on *Andropogon gerardi,* Stillwater, Okla., 1941 (Chester); *V, A. graminea* (Sacc.) Sprague and A. G. Johnson on *Cynodon dactylon,* west of Davis, Calif., May 17, 1935 (O.S.C. 10,910-A); *W, A. graminea* on *Bouteloua gracilis,* Mandan, N. Dak., June 20, 1941 (B.P.I. 80,578); *X, A. sorghina* Sacc. on *Sorghum vulgare,* Madison, Ga., Aug. 19, 1943 (C. L. Lefebvre).

FIG. 2.—Pycnospores of species of *Ascochyta* on Gramineae in the section Ascochytella: *A, Ascochyta subalpina* Sprague and A. G. Johnson on *Distichlis stricta,* Marion, Mont., July 7, 1936 (type); *B, A. agropyrina* (Fairman) Trotter on *Elymus virginicus,* Mandan, N. Dak., June 13, 1941; *C, A. agropyrina* on *Agropyron subsecundum,* Mandan, N. Dak., June 13, 1941; *D, A. agropyrina* on *Agropyron trichophorum,* Mandan, N. Dak., June 13, 1941; *E, A. agropyrina* on *Agropyron subsecundum,* Pullman, Wash., 1941;

F, A. hordei Hara on *Holcus lanatus,* Bingen, Wash., Feb. 26, 1935 (O.S.C. 8,081); *G, A. hordei* on *Hordeum vulgare,* High Prairie, Wash., March 20, 1939 (O.S.C. 771); *H, A. hordei* from pure culture obtained from preceding collection; *I, A. avenae* (Petr.) Sprague and A. G. Johnson on *Avena byzantina,* Troutdale, Oregon, March 13, 1935 (O.S.C. 10,243); *J, A. missouriensis* Sprague and A. G. Johnson on *Panicum virgatum,* Wilton, N. Dak., Sept. 15, 1941 (B.P.I. 80,279) (type).

A TAXONOMIC CONSIDERATION OF TWO CHEIROSPOROUS GENERA, CHEIROMYCES AND PEDILOSPORA

Samuel C. Damon

(with 2 figures)

The genus *Cheiromyces* has recently been reviewed by Martin (7) at which time he added a new species to the genus. The present paper is an attempt to clarify the generic concept as it was conceived by Berkeley (1) in his original description and as it has been reinterpreted by Höhnel (6) and Martin (7). Although material of Berkeley's type specimen has not been seen, drawings and descriptions of it sent to Dr. Martin by Dr. G. R. Bisby have been consulted. These together with an authentic specimen in the Curtis herbarium and material in the Morgan collection at the State University of Iowa allow a relatively complete understanding of the type species.

Berkeley's statement that the conidia of *Cheiromyces* are nonseptate was due either to observation of immature spores or to failure to see the septa. The spores are cheiroid and multiseptate, as evidenced by Bisby's notes on specimens other than the type in the Berkeley collection and by the reports of other authors who have studied these collections. The essential point of difference between the view presented here and the opinions of Höhnel and of Martin arises from the interpretation of the manner in which the spores are formed.

On the basis of the available evidence, the spores of *Cheiromyces stellatus* Berk. are formed in the following manner. The terminal cell of the conidiophore proliferates in an antler-like fashion forming more or less parallel branches. After these branches have formed the spore becomes multiseptate with one or more septa formed in each branch. In most cases a septum is formed at the base of each branch thus leaving the branches attached to a single basal cell. This is what I call branch by branch spore formation in contrast to spore formation in species related to *Cheiromyces digitatus* Martin. In such species, the terminal cell of the conidiophore divides. From the division of this cell the branches of the spore arise by succeeding divisions of the cells formed by the first division of the terminal cell. This is what I call branch formation in a cell by cell manner. The drawings in Martin's paper bear out this interpretation of spore formation in his species. Comparison of these with the drawings of spore formation in *C. stellatus* presented below (FIG. 1, A, B), graphically illustrates this slight but important difference in spore formation.

A further distinction between spores in *C. stellatus* and *C. digitatus* is that the branches are formed in three planes in Berkeley's species and in two in Martin's species. For this reason, I believe that *C. digitatus* should be removed to *Speira.*

This brings up the question of relationship between *Cheiromyces* and *Speira.* In Saccardo's first summary (12) three species, *S. punctulatus, S. Kummeri,* and *S. erumpens,* have descriptions suggesting a sporodochial type of conidiophore arrangement. *Speira,* therefore, in current usage includes species having conidiophores borne in sporodochia as well as species characterized by effuse growth. It is to the sporodochial group that *C. digitatus* Martin belongs, and it should be called **Speira digitata** (Martin *) comb. nov. It is worthy of note at this point that the type species, *S. toruloides* Corda (Icones Fungorum 1: 9, fig. 140. 1837), is illustrated as effuse and properly belongs in the Dematiaceae. This apparent inconsistency of having effuse and sporodochial species in one genus is, however, by no means unusual in the Moniliales. There are excellent examples of similar situations in well-known genera. One striking illustration is the genus *Fusarium* in which *F. lini* is characterized by sporodochia while a closely related species, *F. oxysporum,* has an effuse growth. The genus is placed in the Tuberculariaceae although many species have a characteristically effuse growth. *Penicillium claviforme* is another example. It is a species characterized by coremia in a genus universally considered to belong to the Moniliaceae. Species of *Cephalosporium* possessing coremia were formerly separated in the genus *Tilachlidium* in the Stilbaceae until the coremiate character was found to be too unstable. The genus *Sporoschisma,* in the Dematiaceae, includes the species, *S. paradoxa,* although the phialides of that species are borne in coremia. Furthermore, environmental conditions have often been found to be the decisive factor in the formation of these pseudo-fructifications. Such is the case with *Fusarium* and the *Tilachlidium-Cephalosporium* group. Instances of this sort serve to illustrate the inadequacy as well as the artificiality of some of the criteria used in familial and generic distinctions in the Moniliales and point up the need for a sweeping revision of this group.

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* *Cheiromyces digitata* Martin (7).

The history of *Cheiromyces* is presented in some detail by Martin (7), but a brief synopsis of it is repeated here. *Cheiromyces* was first described by Berkeley (Int. Crypt. Bot. p. 312. 1857) with drawings of non-septate, cheiroid spores. These figures appear on the packet of the type species, *C. stellatus,* in the Kew Herbarium and are reproduced below (FIG. 1, e). There is a second specimen in the Berkeley herbarium labelled *C. Beaumontii* (FIG. 1, c, d), collected in Alabama by Beaumont. Later, this species was considered the same as *C. stellatus* by Berkeley because he referred to it as one of the two collections of that species when he discussed it in Grevillea (2). Furthermore, there is no reference in the literature to the publication of *C. Beaumontii* as a separate species and there appear to be no major differences between the two specimens. *C. Beaumontii* persists as an herbarium name applied to specimens distributed by Curtis with his herbarium and to *No. 762* of North American Fungi distributed by Ellis. The name has no standing and must be considered a *nomen nudum* which has been applied to *C. stellatus.* The specimen at Kew labelled *C. Beaumontii* may, on the other hand, be considered a substitute type for *C. stellatus* since neither Höhnel (6) nor Bisby (3) was able to find a fungus on the Berkeley specimen labelled *C. stellatus.* For practical purposes, however, the specimen in North American Fungi may be used to indicate the nature of the fungus. This view differs from that of Höhnel (6) who, although he had not seen the Berkeley specimen of *C. Beaumontii,* concluded that it was both specifically and generically different from *C. stella-*

tus, and erected for it the genus *Cheiroconium,* placing it in the Melanconiales. Martin (7) has concurred in this view and would assign the specimen, N.A. Fungi *No. 762,* to *Cheiroconium.* After examination of three specimens in the Morgan collection referred to *C. tinctus,* of N.A. Fungi *No. 762,* and of the specimen in the Curtis herbarium labelled *C. Beaumontii,* I am led to the conclusion that they are all specimens of *C. stellatus. Cheiroconium* Höhnel is, therefore, a synonym of *Cheiromyces* Berkeley. This is further supported by the fact that the conidiophores are arranged in sporodochia and not in acervuli as stated by Höhnel. The location of sporodochia in crevices of decorticated wood has led to this misinterpretation of their nature.

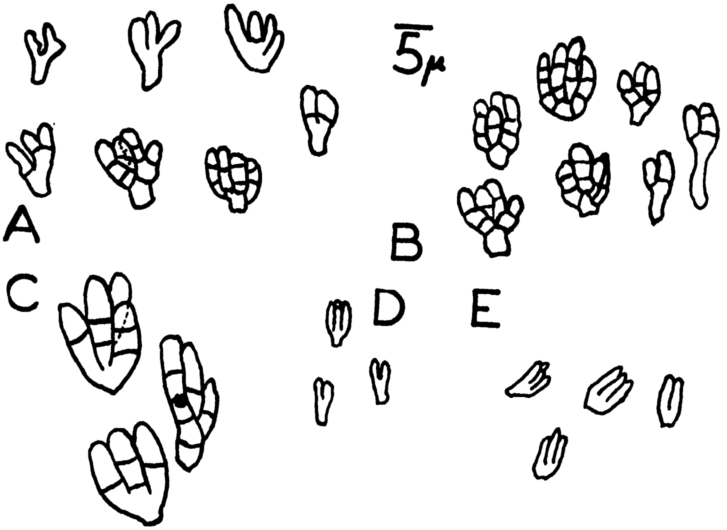

FIG. 1. A. Developing spores of *C. stellatus,* B. Mature spores of *C. stellatus,* C. Bisby's drawings of spores of *C. Beaumontii* (in herb. Berk.), D. Reproduction of Berkeley's drawings of *C. Beaumontii,* E. Reproduction of Berkeley's drawings of *C. stellatus.*

Since the publicaton of Berkeley's species, four others have been added to the genus. Peck (9) described *C. tinctus,* Höhnel (5) described *C. speiroides,* Ellis and Everhart (4) described *C. comatus,* and Martin (7) described *C. digitatus.* Peck's species differed from *C. stellatus* by having a bluish cast to the spores when examined microscopically. Until the type specimen can be seen there is no reason to invalidate the species. After describing *C. speiroides,* Höhnel later removed it to the monotypic genus, *Cheiromycella,* because of the light color of the hyphae of the sporodochium. Examination of a slide of this species at the Farlow Herbarium revealed that the spores are dark-colored and similar in appearance to those of *C. stellatus.* Because of the similarity of spore characters, I include this species in *Cheiromyces* despite the light-colored hyphae. *C. comatus* E. & E. was transferred to *Exosporium* by Höhnel (5). This specimen has not been seen, but the original description supports Höhnel's view. A critical examination of some of the species now included in *Speira* may justify their removal to *Cheiromyces,* but sufficient material is not available at this time to permit this.

Cheiromyces Berkeley, Int. Crypt. Bot. 312. 1857.

Cheiroconium Höhnel, Sitz.-ber. Akad. Wien **119**: 664. 1910.
Cheiromycella Höhnel, Sitz.-ber. Akad. Wien **119**: 664. 1910.

Sporodochia dark, punctiform or hysteriform, scattered, superficial or sunken in crevices of substrate; conidiophores short, hyaline, sometimes branched; conidia acrogenous or subacrogenous, cheiroid, dark-colored, branches of spore attached to a single basal cell, conidia formed in a branch by branch manner, formed in three planes.

Type Species: Cheiromyces stellatus Berkeley, Int. Crypt. Bot. 312. 1857. (also discussed in Grevillea **3**: 97. 1875.)

Key to Species

Spores light olivaceous to dark brown microscopically............. *stellatus*
Spores with bluish cast microscopically.......................... *tinctus*

CHEIROMYCES STELLATUS Berkeley, Int. Crypt. Bot. 312. 1857.

Cheiromyces Beaumontii B. & C., *nomen nudum* in Kew herb. and Curtis herb.
Cheiroconium Beaumontii (B. & C.) Höhnel, Sitz.-ber. Akad. Wien **119**: 664. 1910.
Cheiromyces speiroides Höhnel, Ann. Myc. **1**: 408. 1903.
Cheiromycella speiroides (Höhnel) Höhnel, Sitz.-ber. Akad. Wien **119**: 664. 1910.

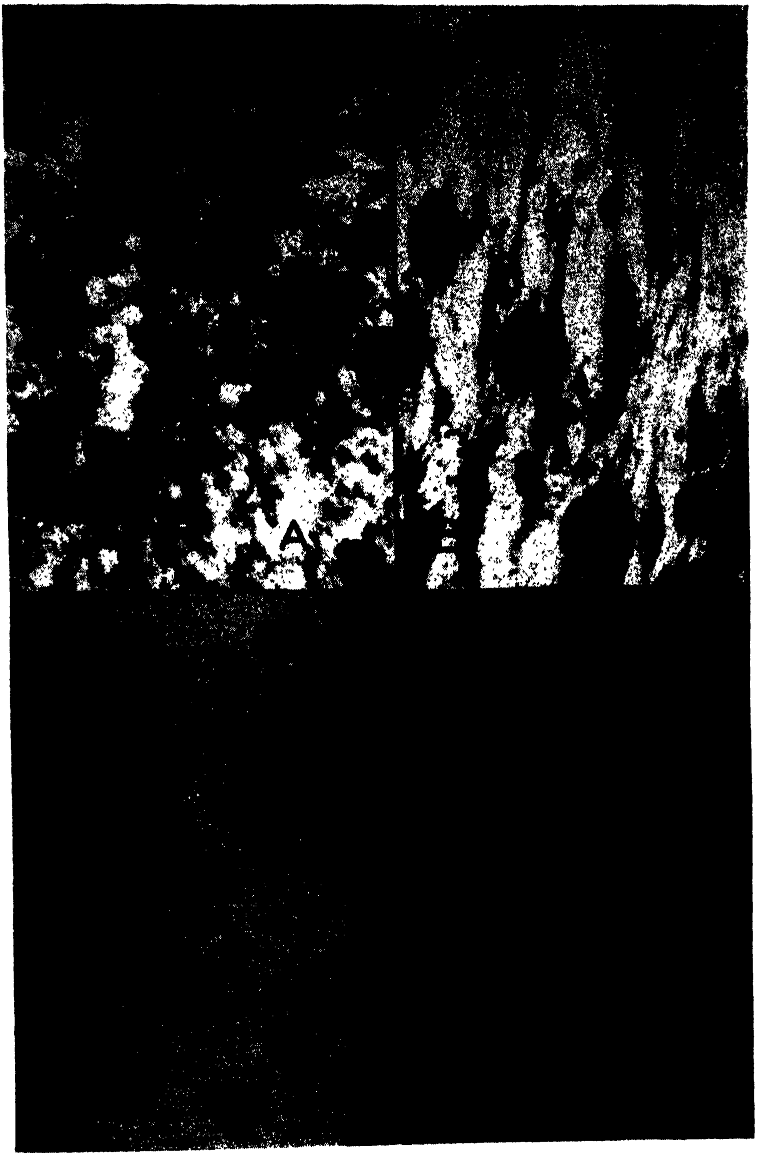

FIG. 2. A. Punctiform sporodochia of *C. stellatus*, × 10, B. Hysteriform sporodochia of *C. stellatus*, × 10, C. Spores of *C. Beaumontii* (in herb. Curt.), D. Spores of *C. stellatus* (in herb. Morg.).

Sporodochia black punctiform or hysteriform, 0.05–1.5 mm. in diameter, hysteriform type often cracking when dry to give the appearance of hysterothecia; conidiophores arising from a compact central mass, hyaline, simple or branched; conidia acrogenous or subacrogenous on conidiophores or their branches, cheiroid, branches usually formed in three planes, 2–6 branches arising from a single basal cell, multiseptate, 11–16 × 6–11 μ, olivaceous in color when mature.

This fungus has been found on decorticated wood of various species.

CHEIROMYCES TINCTUS Peck, Bot. Gaz. **5**: 35. 1880.

Sporodochia black, scattered, hysteriform; conidia 12–20 μ long, one to three septate, often slightly constricted at the septa, having a bluish tint microscopically.

This species has also been found on decaying wood. It is quite possible that *C. tinctus* may be the same as *C. stellatus*, but the color of the spores microscopically and their size separate the two. Examination of the type specimen of *C. tinctus* is necessary before a final decision can be made.

Another cheirosporous species which may be considered at this time is *Tetracrium incarnatum* Martin (**8**). This species was isolated by Martin from dead *Opuntia* stems upon which it formed pale pink sporodochia. The conidia have digitate branches arising from a basal cell in the manner of *Cheiromyces*. That it cannot belong to *Tetracrium* is evident after examination of illustrations of spores of that genus given by Petch (**10**). The species cannot be included in *Cheiromyces* because both hyphae and spores are light-colored, but it may be placed in *Pedilospora* Höhnel despite the sporodochial habit of the conidiophores of this species. Rogers (**11**) has studied an example of Höhnel's species and his drawings of the ontogeny of the conidia show such a striking similarity to those of Martin's *T. incarnatum* that to erect a new genus for Martin's species based solely upon the doubtfully valid character of sporodochial habit would, in my opinion, be a needless multiplication of names. I propose, therefore, to transfer *Tetracrium incarnatum* Martin to *Pedilospora*.

Pedilospora incarnata (Martin) comb. nov.

Tetracrium incarnatum Martin, Pac. Sci. 2: 75. 1948.

Sporodochia pulvinate, 0.4–0.8 mm. in diameter in nature, often 1–4 mm. in culture, becoming confluent, white at first becoming flesh-colored and finally cinnamon; conidia acrogenous on elongate conidiophores which are attenuated below the point of conidial attachment; conidia formed in a branch by branch manner, the 3–8 branches attached to a single basal cell, 40–50 μ × 10–20 μ.

The material from which the species was isolated was collected by G. W. Martin on South Seymour Island in the Galapagos group.

The fungus grows in culture on the usual artificial media as a moist, deep pink, submerged colony and shows poor development. There is no aerial mycelium except where the colony comes in contact with the edge of the plate or tube where a slight fringe of aerial hyphae is formed. On the other hand, it develops aerial hyphae and sporodochia when grown on media in which pieces of cornstalk or *Desmodium* stalks have been included, and experiments seem to indicate that sporodochium production can be correlated with the solid substratum in the medium. The color of the fungus in culture has been found to be paler than in nature but this character varies with the medium used.

P. incarnata can be distinguished from *P. parasitans* by its larger spores and tufted habit. It will also be noted that the genus is characterized by having conidiophores with a slight attenuation below the attachment of the conidia as well as having cheiroid spores.

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The author wishes to express his thanks to Dr. Walter H. Snell for the loan of the specimen of *C. Beaumontii* from the Curtis herbarium and to Dr. G. W. Martin for helpful suggestions and criticism during the preparation of this paper and for reading the manuscript.

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THE SYNONYMITY OF PYTHIUM DISSOTOCUM DRECHSLER AND PYTHIUM PERIGYNOSUM SPARROW[1]

JOHN T. MIDDLETON

Pythium dissotocum Drechsler (1930) was originally described from roots of *Saccharum officinarum* L. from Louisiana and may be characterized as a typical member of the genus *Pythium* possessing filamentous sporangia, spherical and smooth-walled oogonia, stalked antheridia, and spherical, smooth-walled, aplerotic oospores. *P. perigynosum* Sparrow (1936) was originally described from hemp-seed bait placed in an English water course containing plant debris and may also be characterized a typical member of the genus *Pythium* as set forth above. From the original and only published account of *P. perigynosum,* it appears that the fungus should be considered synonymous with the earlier-described *P. dissotocum.* It is the purpose of this report to establish that synonymity.

Ten years after the original description of *P. dissotocum,* Drechsler (1940) published supplementary information and illustrative material for the species. The writer (1943) figured and discussed *P. dissotocum* in a monograph of the genus *Pythium* and established the synonymity of this species with *P. oryzae* Ito and Tokunaga (1933). The writer was unaware of the existence of *P. perigynosum* at that time and was therefore unable to include a discussion of it. The description of *P. perigynosum* was subsequently brought to his attention through the courtesy of F. K. Sparrow, Jr., who wrote (in correspondence, in 1943): "your figures of the species [*P. dissotocum*] make me more sure than ever of the identity of the two [*P. dissotocum* and *P. perigynosum*]." Observations of the type culture of *P. dissotocum* and of several additional isolates secured by the writer, and a critical perusal of the original description of *P. perigynosum,* reveal the similarity of the two species, despite the unavailability of cultures of the latter.

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[1] Paper No. 636, University of California Citrus Experiment Station, Riverside, California.

The salient features of the two species, *Pythium dissotocum* Drechsler (1930) and *P. perigynosum* Sparrow (1936), are presented in table I.

TABLE I

COMPARISON OF THE SPECIES

Fungus parts	*Pythium dissotocum*	*Pythium perigynosum*
Mycelium	3.5–6.0 μ in diameter	Av. 5.2 μ in diameter
Sporangia	Undifferentiated filamentous; occasionally inflated	Undifferentiated filamentous
Zoospores	Moderate number; cystospore 8–9 μ in diameter	Various numbers; cystospore 10 μ in diameter
Oogonia	Acrogenous or intercalary; subspherical, av. 20.7 μ in diameter; smooth-walled	Usually acrogenous; papillate, spherical or subspherical, av. 15.6 μ in diameter; smooth-walled
Antheridia	Mono- or diclinous, 1–5, autonomous, usually sessile and adjacent to oogonium	Mono- or diclinous, 1–5, autonomous, usually originating from oogonial stalk and adjacent to it
Oospores	Aplerotic, av. 17.6 μ in diameter; spherical reserve globule av. 8.4 μ in diameter	Aplerotic, av. 13 μ in diameter; spherical reserve globule av. 8 μ in diameter

DISCUSSION

The descriptions presented in table I readily demonstrate the similarity of the two species. The differences between them seem to possess little taxonomic significance, consisting as they do in the dimensions of oogonia and oospores, which vary not only within a single species but within a single isolate of a species when the quality of the culture medium employed is altered.

Although Sparrow (1936) reports the oogonia of *Pythium perigynosum* as usually terminal and frequently papillate, and does not mention their intercalary nature, he presents one figure in which an intercalary oogonium is shown. The oogonia of *P. dissotocum* are typically nonpapillate, although occasional papilla-like structures are observed. These papilla-like structures are usually the initial development of later hyphal growth which ultimately results in the production of typically intercalary oogonia. It is therefore possible that Sparrow's reference to the apical papilla in *P. perigynosum* may be interpreted as indicating a tendency for some of the oogonia to become intercalary, as is the case in *P. dissotocum.*

Taxonomic significance can be attached (Middleton **1943**) to the type of sporangium, oogonium, and oospore, and the type and disposition of the antheridium. When these categories are considered, the identity of *Pythium dissotocum* and *P. perigynosum* becomes strikingly apparent. It is proposed that the two species be designated as synonymous, and that inasmuch as *P. dissotocum* has priority, it be maintained as the valid binomial. The species would then include the following:

PYTHIUM DISSOTOCUM Drechsler, Wash. Acad. Sci. Jour. **20**: 398–418. 1930.

Pythium oryzae Ito and Tokunaga. Hokkaido Imp. Univ., Faculty Agr., Jour. **32**: 201–233. 1933.

Pythium perigynosum Sparrow. Linnean Soc. London, Jour. Bot. **50**: 417–478. 1936.

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LITERATURE CITED

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ENTOMOGENOUS SPECIES OF AKANTHOMYCES, HYMENOSTILBE AND INSECTICOLA IN NORTH AMERICA[1]

E. B. Mains

(WITH 26 FIGURES)

The form-genus *Akanthomyces* was established by Lebert (6) in 1858 for a species, *A. aculeata,* found on a moth in Europe. Apparently because it was published in a zoological journal it was overlooked for a long time in mycological literature. In 1892, Thaxter (20) used the name *Acanthomyces* for a genus of the *Laboulbeniaceae.* He later (21, 22) corrected this giving the name *Rhachomyces* to the genus of the *Laboulbeniaceae* and suggested that *Akanthomyces aculeata* was probably identical with *Isaria sphingum* Schw.

In 1931 Petch (10) proposed the form-genus *Hymenostilbe* for *H. muscarium,* a species on flies, as well as for *Isaria arachnophila* Ditm. and for the conidial stages of several species of *Cordyceps,* namely *C. dipterigena, C. lloydii* and *C. australis.* Later (13) when he discussed *Akanthomyces aculeata,* he expressed doubt whether *Hymenostilbe* could be maintained as distinct from *Akanthomyces.* However, he (15) finally concluded that they are distinct, the most important difference being that the conidia of *Akanthomyces* are catenulate and those of *Hymenostilbe* solitary on the phialide. This is a distinction which is commonly used in the separation of genera in the Fungi Imperfecti.

Akanthomyces and *Hymenostilbe* are form-genera of the *Stilbaceae.* They produce synnemata which are more or less cylindric often becoming somewhat attenuated upward. The synnemata are composed of more or less parallel longitudinal conidiophores usually forming a compact bundle. The longitudinal hyphae produce phialides at their terminations, specially in the upper portions of the synnema (FIG. 16A). However, most of the phialides are produced either as lateral cells (FIGS. 16B, 18B, 19B, 24A) or frequently as terminal cells of short lateral branches produced along the entire lengths of the outer hyphae of the synnema (FIGS. 17A, 23A, 25A, 26A). This results in a hymenial layer which covers the surface of the synnema. In most species there is abundant production of phialides resulting in a compact hymenial layer (FIG. 3). In some species the phialides are scattered and well separated from each other (FIG. 6).

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[1] Paper from the Herbarium and the Department of Botany of the University of Michigan. This paper is published as excess pagination. The cost has been paid by the Herbarium of the University of Michigan.

The phialides either have no well differentiated sterigma or their apices are prolonged into short acute sterigmata. In *Akanthomyces* conidia are produced in succession at the apex of the phialide and adhere to form a chain of spores (FIGS. 16A, 18A). The strength of this adherence varies due to conditions at the time of production. It seems doubtful that the spore production of the phialides of species of *Hymenostilbe* is limited to one conidium. If only one conidium is produced collapsed phialides would be expected far more commonly than is the case. It is probable that several conidia, which do not adhere, are produced in succession and consequently spores are found singly on the phialides. If this is so the distinction between the genera is slight. On account of the variation of adherence of spores it may not always be possible to determine accurately the catenulate character for a species known from a few specimens. In this treatment the species in which catenulate spores have been observed are placed in *Akanthomyces.* Species in which conidia have been observed only singly on the phialides are placed in *Hymenostilbe.* If eventually the genera are combined the generic name *Akanthomyces* should be employed for the group.

Three species have been studied which have the catenulate character of *Akanthomyces* but differ in that the hymenium is limited to the upper part of the synnema and the lower portion is sterile forming a stipe (FIGS. 8, 10). Unlike *Akanthomyces* the outer longitudinal hyphae of the synnema do not produce lateral phialides or lateral branches bearing phialides. The hyphae diverge in the

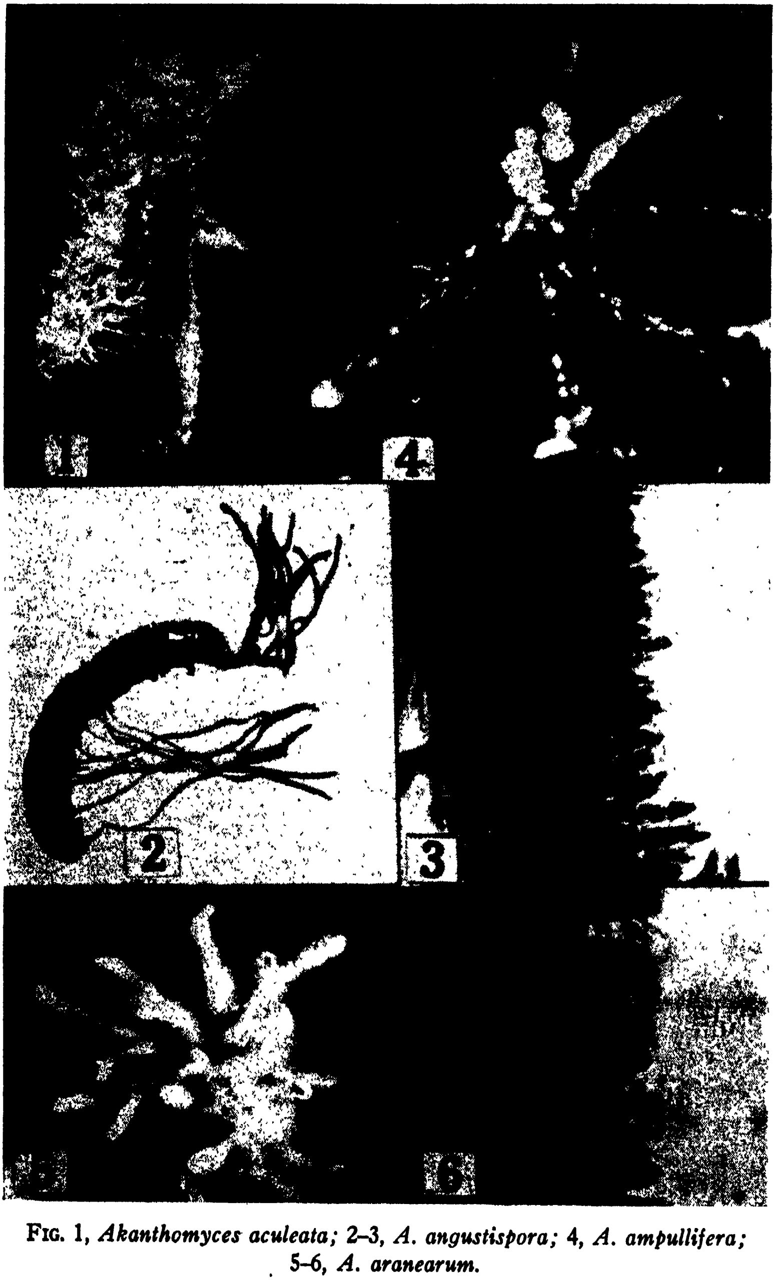

FIG. 1, *Akanthomyces aculeata;* 2–3, *A. angustispora;* 4, *A. ampullifera;* 5–6, *A. aranearum.*

upper portion of the synnema and repeatedly branch more or less dichotomously. The phialides are terminal on the branches (FIGS. 20A, 21A, 22A). The hyphae are closely packed forming a compact enlargement of the synnema which is covered by a compact palisade layer of phialides over the upper portion of the synnema. The structure of the synnema is similar to that in *Stilbum* which, however, has conidia covered with a mucus and produced singly. *Coremium* as typified by *Coremium glaucum* Link has a terminal sporogenous head and catenulate spores but the head is composed of a loose mass of conidiophores which are terminated by whorls of phialides. The three species are placed in a new genus, for which the name *Insecticola* is proposed.

This study is based on North American collections in the Farlow Herbarium (FH),[2] the Herbarium of the New York Botanical Garden (NY), the Mycological Collections of the U. S. Bureau of Plant Industry, Washington, D. C. (W) and the Herbarium of the University of Michigan (M). Several species represented only by collections made by Thaxter in Trinidad are included since they may be expected in the West Indies.

The species treated in this paper are found on orders of the *Insecta* and on the *Arachnida* as follows.

Lepidoptera	1. *Akanthomyces sphingum*
	7. *Insecticola pistillariaeformis*
Coleoptera	2. *Akanthomyces angustispora*
Diptera	3. *Akanthomyces ampullifera*
	8. *Hymenostilbe dipterigena*
Orthoptera	5. *Insecticola clavata*
	6. *Insecticola fragilis*
Homoptera	9. *Hymenostilbe lecaniicola*
	10. *Hymenostilbe aphidis*
Hymenoptera	11. *Hymenostilbe formicarum*
	12. *Hymenostilbe camponoti*
	13. *Hymenostilbe sphecophila*
Arachnida	4. *Akanthomyces aranearum*
	14. *Hymenostilbe verrucosa*

AKANTHOMYCES Leb. Zeitsch. wissen. Zoologie 9: 447. 1858

Synnemata cylindric often somewhat attenuated upward, composed of longitudinal, more or less parallel, closely compacted hyphae (conidiophores); phialides in a hymenial layer covering the synnema or in *A. areanearum* somewhat scattered over the surface, produced as terminal cells of short lateral branches from the outer hyphae or arising as lateral cells or buds of the outer hyphae of the synnema, or less frequently terminating the hyphae of the synnema, ellipsoid, obovoid or cylindric usually more or less acute at the apex and terminating in a short sterigma, smooth or roughened; conidia one-celled, hyaline, smooth, catenulate.

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[2] The writer is indebted to W. Lawrence White, Rolf Singer, Donald P. Rogers and John A. Stevenson for the loan of specimens.

Type species: *Akanthomyces aculeata* Leb.

The genus *Akanthomyces* has hitherto been limited to one species, *A. aculeata.* In this study three additional species, *A. ampullifera, A. angustispora* and *A. aranearum* are recognized. *A. aculeata* develops on the adults of lepidopterous species of insects. *A. ampullifera* occurs on craneflies. *A. angustispora* is described from a recent collection on a coleopterous larva from Tennessee received from F. T. Wolf and *A. aranearum* appears to be fairly widespread on spiders.

1. AKANTHOMYCES ACULEATA Leb. FIGS. 1; 16A–C

Zeitsch. wissen. Zoologie **9**: 447. 1858

?*Isaria sphingum* Schw. ex Fr. Sys. Mycol. **3**: 275. 1832.

?*Hymenostilbe sphingum* (Schw.) Petch, Trans. British Mycol. Soc. **16**: 217. 1932.

?*Akanthomyces sphingum* (Schw.) Petch, Trans. British Mycol. Soc. **27**: 82. 1944.

Yellowish mycelium more or less covering adult moths and attaching them to the substratum; synnemata arising from various parts of the insect, often developing from conical masses of mycelium, terete, narrowing upward, very variable in length, 1–8 mm. long, slender, 0.1–0.5 mm. thick, yellowish, composed of longitudinal somewhat interwoven hyphae; phialides densely compacted into a layer covering the synnema, terminating the hyphae or arising directly as lateral cells from the outer hyphae of the synnema or terminating short lateral branches, subcylindric or narrowly ellipsoid, 6–16 × 2.5–4 μ, narrowing above to an acute apex terminated by a short sterigma up to 4 μ long, smooth; conidia broadly ellipsoid or obovoid often acute at the lower end, 3–6 × 2–3 μ, smooth, hyaline catenulate.

Connecticut: On *Amphipyra pyramidoides* (*Pyrophila pyramidoides*), New Haven, Nov. 1888, R. Thaxter, Reliq. Farl. 661.

Washington: On moth, Baker Lake, Aug. 14, 1941, A. H. Smith, 17893 (M).

Ontario: On moth, Deep Creek, Sept. 4, 1947, A. H. Smith, 26556 (M).

Salvador: On moth, Santa Techla, July 1, 1943, Padre Anton Kovar (W).

This species has usually been reported as *Isaria sphingum* Schw. Petch (**14**) has decided that *Akanthomyces aculeata* is a synonym and has made the combination *Akanthomyces sphingum.* He has considered the species to be very variable both in the development of synnemata and in the shape and size of conidia. From the description given by Schweinitz (**16**) it is not possible to determine the application of the name *I. sphingum* with certainty since microscopic characters of the fungus are not described. Through the kindness of Dr. Francis F. Pennell a specimen determined as *Isaria sphingophila* Schw. in the Schweinitz collection of the Academy of Natural Sciences of Philadelphia has been available for study. This is probably the type of *I. sphingum.* Schweinitz in his Synopsis of North America Fungi (**17**) under no. 3055 *Isaria sphingophila* L. v S., Lk. refers to no. 1298 in his Synopsis Carolinae (**16**) where *I. sphingum* was described. Link (**7**) apparently substituted the name *I. sphingophila* for *I. sphingum.* The specimen from the Schweinitz collection bears only the data "Salem-Beth." It consists of an adult moth attached to a twig. A thin layer of a cream-colored mycelium covers the insect and attaches it to the substratum. The synnemata arise from conical masses of mycelium scattered over the insect. They are pale brown and vary considerably in length from a few millimeters to one centimeter, are terete, 0.2–0.5 mm. thick, and gradually attenuated upward. Phialides and spores were not observed from an examination of several synnemata. The specimen was probably effete when collected. Unfortunately, therefore, it is not possible to determine the microscopic characters of *Isaria sphingum.*

Petch (**15**) gives *Isaria floccosa* Fr. and *I. strigosa* Fr. as synonyms of *Akanthomyces sphingum.* Fries (**3**) published these in the Systema Mycologicum immediately preceding *I. sphingum.* They are described as developing on larvae and pupae of the Lepidoptera whereas *I. sphingum* was said to occur on adults. Their identity cannot be determined with any certainty. All the collections seen by the writer have been on adult lepidopterous insects. Petch (12) also includes *Isaria pistillariaeformis* Pat., *I. surinamensis* Voss., and *Hirsutella clavispora* Petch as synonyms of *Akanthomyces sphingum.* The type of *I. pistillariaeformis* Pat. has been studied and found to be another species (see no. 7) having synnemata with sporogenous heads and sterile stipes. Vosseler (25) describes *I. surinamensis* as having synnemata up to 12 cm. long with short side branches bearing conidia 2 μ in diameter. Speare (18) has suggested that this species is a *Hirsutella.* Petch (12) describes the conidia of *Hirsutella clavispora* as cylindric to clavate, 4–8 × 1–1.5 μ with a mucous coat. Neither *I. surinamensis* nor *H. clavispora* appears to be the species described here. Lebert (6) in establishing the genus *Akanthomyces* describes and illustrates the phialides and conidia of *A. aculeata.* The species as circumscribed here agrees very well with his description. Although it is probable that *Isaria sphingum* is the same species it seems best to use name *Akanthomyces aculeata* which can be applied with greater accuracy.

The Tulasnes (23) when they described *Torrubia sphingum* (= *Cordyceps tuberculata*) suggested that the conidial stage was *Isaria sphingum.* They did not observe an association of perithecial and conidial stages. This connection has been commonly accepted although it is surprising how few times perithecia and conidia have been found associated. Lebert (6) describes *Cordyceps tuberculata* as *Akrophytum tuberculatum* in the same publication as *Akanthomyces aculeata* but does not indicate a connection. Vincens (24) found perithecia associated with minute synnemata bearing catenulate globose conidia 2 μ in diameter. The perithecial connections reported by Petch (12) were with synnemata of *Isaria pisillariaeformis* Pat. Kobayasi (5) records a number of collections of *Cordyceps tuberculata* and *Hymenostilbe sphingum* from Japan and states that he had obtained no collection bearing both perithecia and conidia. While the development of both *Cordyceps tuberculata* and *Akanthomyces aculeata* on adult moths is very similar, the evidence for their connection is by no means conclusive.

2. **Akanthomyces angustispora** sp. nov. FIGS. 2–3; 17A–B

Synnemata simplicia vel ramosa, 8–13 mm. longa, 0.2–0.6 mm. crassa, carnea, ex longitudinalis compaginatis hyphis composita; phialides densum hymenium formantes, oblongae vel ellipsoideae, 6–14 × 3–4 μ, superne acutae; conidia anguste clavata, 4.5–6 × 1.2–1.4 μ, catenulata.

Ex larva scarabaei, Nashville, Tenn., Jan. 15, 1950, F. T. Wolf, 473, in Herb. Univ. Mich.

Synnemata arising from the body and head of a coleopterous larva, simple or branched, 8–13 mm. long, 0.2–0.6 mm. thick, slightly pulverulent, flesh colored, composed of compacted longitudinal hyphae 2.5–3.5 μ wide; phialides forming a dense palisade layer covering the synnema, arising from the ends of the longitudinal hyphae or as terminal cells of short lateral hyphal branches from the longitudinal hyphae of the synnema, oblong or narrowly ellipsoid, 6–14 × 3–4 μ narrowing above into an acute apex terminated by a short sterigma, smooth; conidia narrowly clavate, 4.5–6 × 1.2–1.4 μ, smooth, hyaline, catenulate.

From a coleopterous larva, Percy Warner Park, Nashville, Tenn., Jan. 1950, F. T. Wolf, 473, type.

The synnemata from the body of the larva are all simple. From the head there arises a short main stem whose growth may have been stopped by an injury. Two branches arise from the apex of the stem, each bearing several secondary branches. The phialides which either develop directly laterally from the longitudinal hyphae or as terminal cells from short lateral branches from the longitudinal hyphae (FIG. 17A) form a compact layer covering the synnema. The conidia were observed in short chains of 2 or 3 spores.

3. **Akanthomyces ampullifera** (Petch) comb. nov. FIGS. 4; 18A–C

Hymenostilbe ampullifera Petch, Trans. Brit. Myc. Soc. **21**: 55. 1937.

Mycelium slightly covering the host and attaching it to the substratum; synnemata from various parts of the insect, up to 3 mm. long, very slender, 120–144 μ thick below, narrowing to an acuminate apex, brown, white from pulverulent masses of adhering spores, composed of longitudinal parallel hyphae; phialides arising from the mycelium and directly from the outer hyphae of the synnemata as lateral projections forming a moderately dense palisade layer covering the synnema, ellipsoid to short cylindric, 10–15 × 3–5 μ, narrowing above into an acuminate apex with a short sterigma, smooth; conidia narrowly cylindric 5.2–9 × 1.1–2 μ slightly narrowing but rounded at the ends, smooth, hyaline, catenulate.

North Carolina: On *Limonia fusca* (*Dicranomyia pubipennis*), Cranberry, R. Thaxter, Aug. 1887, type (FH 727); on gnats, Cranberry, R. Thaxter, 1887 (FH 6254).

In his description of this species Petch does not mention the catenulate development of the conidia. This is very pronounced (FIG. 18A) and causes the spores to remain attached to the synnemata in large masses (FIG. 4). In microscopic mounts chains of as many as six spores were found attached to sterigmata. The species therefore belongs in *Akanthomyces*.

4. **Akanthomyces aranearum** (Petch) comb. nov. FIGS. 5–6; 19A–C

Hymenostilbe aranearum Petch, Trans. British Mycol. Soc. **16**: 221. 1932.

Hosts covered and attached to the substratum with a yellowish brown, asperulate mycelium; synnemata arising from all parts of the host, cylindric to clavate, 0.8–10 mm. long, slender, 0.1–0.2 mm. thick, simple or occasionally slightly branched, brown, sometimes pubescent below from short, flexuous, asperulate hyphae, becoming white from conidia adhering on the upper portion, composed of longitudinal parallel hyphae the outer brown and asperulate; phialides somewhat scattered, forming a loose hymenium, arising as lateral cells from the outer hyphae of the synnema, obovoid or ellipsoid 6–12 × 4–8 μ, rounded above and abruptly narrowing into a short sterigma, asperulate; conidia narrowly obclavate, 8–14 × 1.5–3 μ, often acute at the lower end, narrowing upward, rounded or obtuse at the upper end, hyaline, smooth, catenulate.

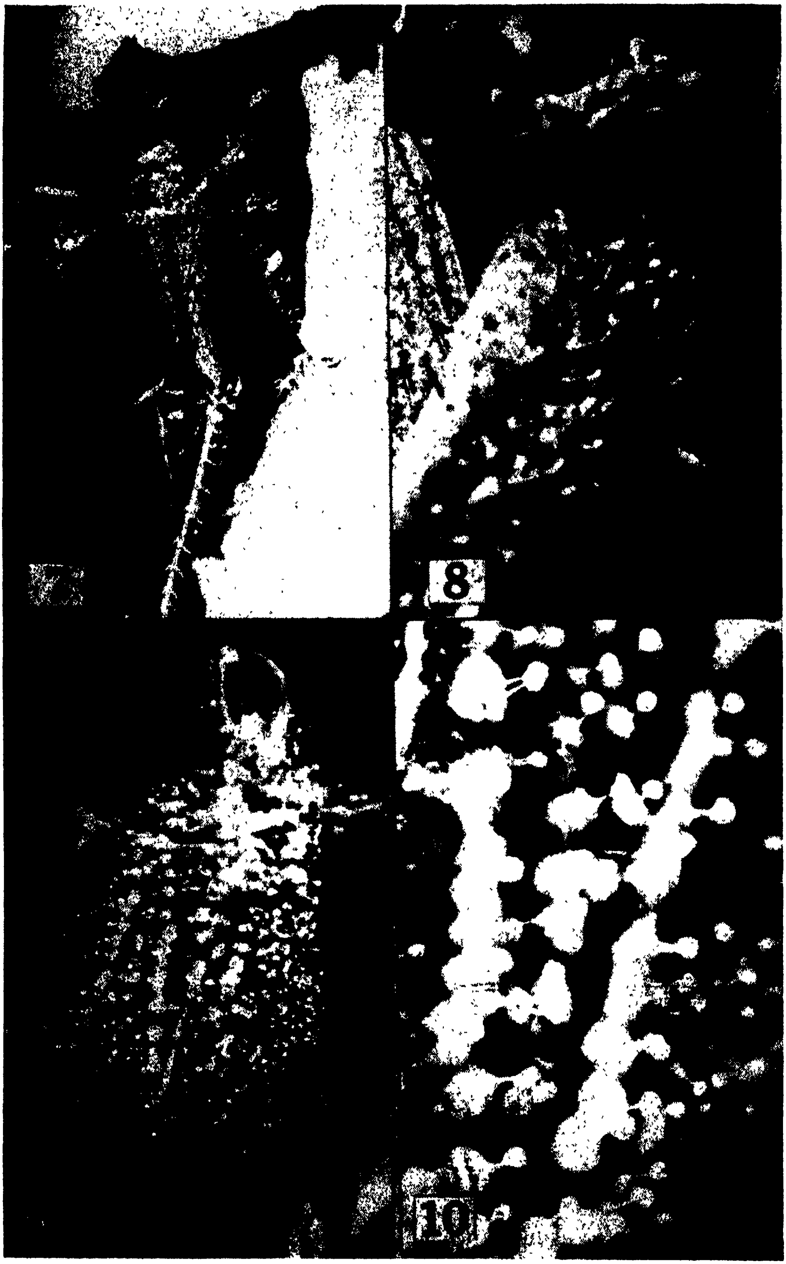
Maine: On spiders, York, Sept. 22, 1892, R. Thaxter (FH 6258); York, Sept. 16, 1896, R. Thaxter (FH 649).

North Carolina: On spiders, Cullowhee, 1887, R. Thaxter (FH 665); Cranberry, July–August 1887, R. Thaxter (FH 4123).

The obclavate shape of the conidia is unusual (FIG. 19C). The conidia are attached to the sterigmata at the broader end and the spores narrow upward (FIG. 19A). The conidia were commonly found in chains and this apparently is the cause of the persistent floccose masses of conidia adhering to the synnemata (FIG. 5). The asperulate condition of the mycelium and outer hyphae of the synnemata is also found in species of *Gibellula* occurring on spiders.

Collections 649, 665 and 6258 were determined by Petch as *Hymenostilbe aranearum,* a species which he (12) described from Ceylon. In his comments he states that in wet weather the conidia of the Ceylon specimens persist in chains. The species therefore belongs in *Akanthomyces.*

Isaria arachnophila Ditmar is probably this species. Ditmar's illustrations (2) indicate that the conidia of his species were probably catenulate since he shows them adhering together in floccose masses on the synnemata in a similar manner to that noted in the collections studied here. Ditmar described the spores as globose "wie bei den übrigen arten dieser Gattungen." Petch (11) states that it seems to have been customary when *Isaria arachnophila* was described to style all small conidia globose and consequently not much weight can be given to that description. Petch applies the name to a species for which he had collections from Southampton, England, stating that it "agrees with Ditmar's figure except that the clava is not covered with loose masses of conidia." He made the combination *Hymenostilbe arachnophila* (Ditm.) Petch. Judging by a collection (No. 6270B) in the Farlow Herbarium determined by Petch as *H. arachnophila* the conidia are obovoid and produced singly (see no. 14). It is difficult to understand why he reached these conclusions unless it was on the basis of the distribution as it was then known. Since the Ceylon species is now known also from Maine and North Carolina it may be expected to occur in Europe. If Ditmar's name applies to either species it would seem more logical to apply it to *Akanthomyces aranearum.* As has been discussed elsewhere (9) the name *Isaria arachnophila* has been used in Europe by several authors for *Gibel-*



FIGS. 7–8, *Insecticola clavata*; 9–10, *I. pistillariaeformis*.

lula leiopus (Vuill.) Mains. Petch (11) also has suggested that if Ditmar's fungus had globose spores as described it might be an *Isaria* form of *Beauvaria densa.* The information available is not sufficient to apply the name with certainty. It is questionable whether there is anything to be gained by disregarding or discounting the information which is given in order to employ the name for one of the fungi now known on spiders.

In collection 4123 of the Farlow Herbarium the synnemata of *A. aranearum* are found associated on some of the specimens with perithecial clavae of *Cordyceps thaxteri* Mains.

Insecticola gen. nov.

Synnemata clavata, stipitata, superis partibus fertilibus; stipites teretes, ex longitudinalis compactis hyphis compositi; superae fertiles partes compactae, ex ramosis hyphis compositae, hymenii phialidium tectae; conidia continua, levia, hyalina, catenulata.

Species typica, *Insecticola clavata.*

Synnemata clavate, stipitate, with the upper portions sporogenous; stipes terete, composed of longitudinal, parallel, closely compact hyphae; the upper fertile portions compact, composed of repeatedly branching hyphae terminated by phialides which form a compact hymenium; conidia one-celled, smooth, hyaline, catenulate.

5. **Insecticola clavata** sp. nov. FIGS. 7–8; 20A–B

Synnemata multa, pallide brunnea, clavata, 0.5–2.0 mm. longa; stipites 60–250 μ crassi, ex longitudinalibus hyphis compositi; superae fertiles partes subcylindricae, obovoideae vel ellipsoideae, interdum furcatae, 300–1500 μ longae, 102–500 μ crassae, hymeniis phialidium tectae; phialides subcylindricae, 17.1–21.4 × 2.8–4.3 μ, superne acutae, brevis sterigmatibus; conidia ellipsoidea vel oblonga, 4.5–8.5 × 2.1–2.5 μ, hyalina, catenulata.

Ex Hapitho, Gainesville, Florida, Nov. 11, 1935, R. E. Bellamy 571 in Herb. Univ. Mich.

Mycelium thin brown partly covering insect; synnemata numerous, arising from various parts of the host, light brown, clavate, 0.5–2.0 mm. long; stipes 60–250 μ thick, composed of longitudinal parallel compact hyphae 2–4 μ wide, the outer brownish; the fertile portions of the synnemata terminal, subcylindric, obovoid or ellipsoid, occasionally furcate, 300–1500 μ long, 102–500 μ thick, covered with a compact hymenium of phialides, often white and pulverulent from adhering masses of conidia; phialides subcylindric, 17.1–21.4 × 2.8–4.3 μ narrowing above to acute apices, terminated by short sterigmata; conidia ellipsoid to oblong, 4.5–8.5 × 2.1–2.5 μ, hyaline, catenulate.

Florida: On *Hapithus agitator quadratus,* moist floodplain forest along Hogtown Creek, 2 mi. N.W. Gainesville, R. E. Bellamy, 571.

This specimen was received from Theodore H. Hubbell of the Museum of Zoology of the University of Michigan. The small synnemata occur scattered on various parts of the insect, specially upon the legs, antennae and between the segments of the body (FIG. 7). The longitudinal parallel hyphae of the stipes diverge outward repeatedly branching to form the upper compact fertile parts of the synnemata. The phialides terminate the branches to form a compact hymenium (FIG. 20A). The conidia were observed in chains of as many as six.

6. **Insecticola fragilis** (Petch) comb. nov. FIGS. 11; 21A–C

Hymenostilbe fragilis Petch, Trans. British Mycol. Soc. **21**: 56. 1937.

Mycelia yellowish white, slightly covering the insects and attaching them to the substratum, bearing terminal or subterminal lateral globoid to ellipsoid verrucose cells on the hyphae; synnemata numerous arising from all parts of the host, clavate, 0.7–1.5 mm. long; the upper portion sporogenous, subgloboid to obovoid, 130–300 μ long, 130–250 μ thick, white, pulverulent and appearing globoid from masses of spores; the stipe 30–90 μ thick, composed of longitudinal parallel closely compacted hyphae; phialides forming a compact hymenium covering the upper portion of the synnema, formed of the terminal cells of repeatedly more or less dichotomously branching hyphae, subcylindric to narrowly clavate, 7–10 × 2.5–3 μ, verrucose in the upper portions; conidia subcylindric 6.5–9 × 1.5 μ somewhat narrowed and rounded at the ends, catenulate.

Trinidad: On an orthopterous larva, Verdant Vale, October 1912, R. Thaxter, type (FH 2581).

Petch (14) states that both the head and the stalk bear a palisade layer of phialides and describes the species in *Hymenostilbe.* An examination of the type shows that the synnemata have sterile stipes composed of longitudinal closely compacted hyphae which diverge above repeatedly branching and ending in the verrucose phialides (FIG. 21A) which form a hymenium over the upper portions of the synnemata. The stipes bear short flexuous hyphae which bear verrucose cells (FIG. 21B) as do hyphae of the mycelium. It is doubtful if these are phialides. They differ considerably in shape and as far as observed do not produce conidia.

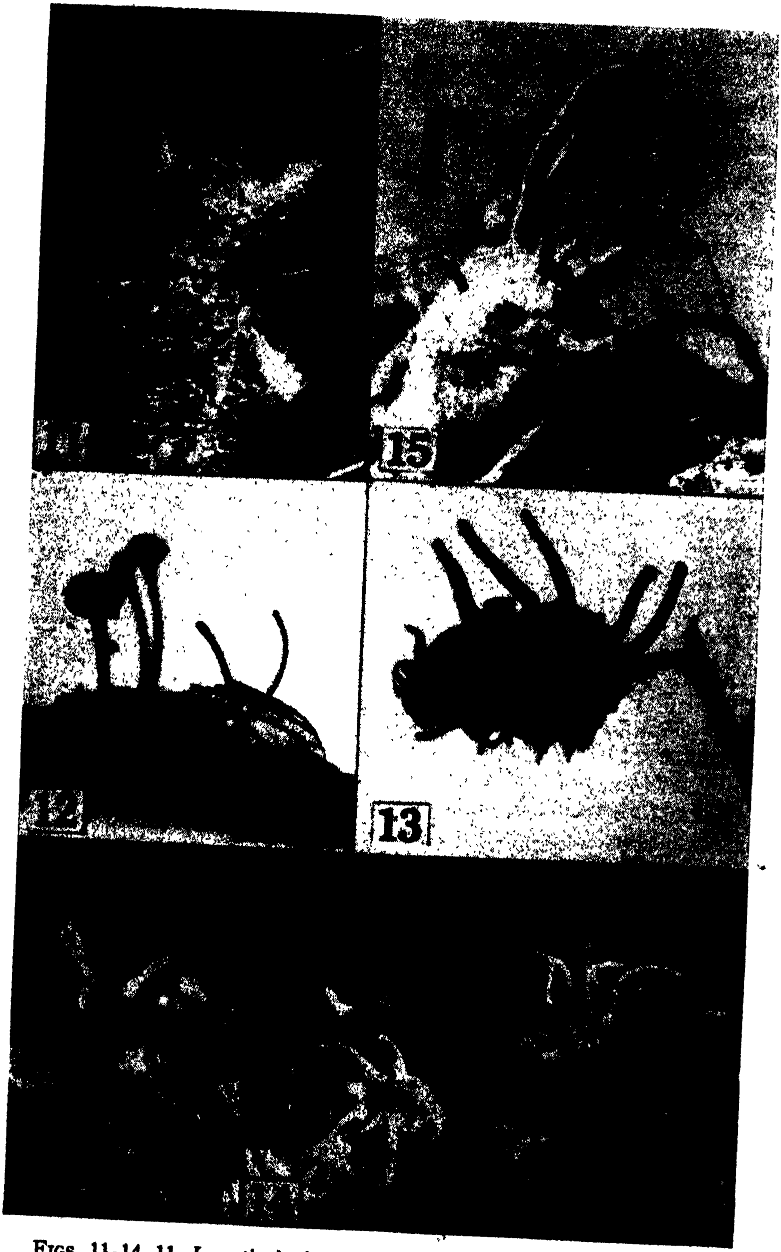
7. **Insecticola pistillariaeformis** (Pat.) comb. nov. FIGS. 9–10; 22A–B

Isaria pistillariaeformis Pat., Bull. Soc. Mycol. France **9**: 163. 1893.

Mycelia whitish, covering adult moths with a thin layer and attaching them to the substratum; synnemata arising from all parts of the moths from bulbose to conic masses of mycelium, clavate, 0.4–1.0 mm. long, stipitate, the upper sporogenous portion subgloboid, ellipsoid or obovoid, 110–200 μ long, 70–170 μ wide, becoming pulverulent with conidia; the stipe 25–50 μ thick, cylindric composed of longitudinal somewhat loosely interwoven hyphae which diverge above repeatedly branching more or less dichotomously to terminate with phialides; phialides forming a compact hymenium over the upper portion of the synnema, subcylindric, 6–10 × 2–3 μ, narrowing above into an acute apex terminated by a short sterigma 2–3 μ long, smooth; conidia fusoid to subcylindric narrowing at the ends, 2.5–5 × 1–1.5 μ, hyaline, catenulate.

Trinidad: On adult moths, Maraval Valley, June 12, 1913, R. Thaxter (FH 2565) and 1912–1913 (FH 2584).

These collections agree well with the type of *Isaria pistillariaeformis* in the Farlow Herbarium. According to the data on the packet it was collected by G. de Lagerheim at Puente de Chimbo in September 1891. The specimen consists of a small moth bearing a few small synnemata arising from a slight whitish mycelium. The synnemata are clavate, 388–525 μ long with the upper sporogenous portion obovoid to subcylindric 165–225 μ long and 75–100 μ thick, and with a stipe 30–40 μ thick. The hyphae of the stipe are longitudinal and loosely interwoven. In the upper sporogenous portion of the synnema the hyphae branch repeatedly and end with the phialides forming a compact hymenium. The phia-



Figs. 11–14, 11, *Insecticola fragilis*; 12–13, *Hymenostilbe dipterigena*; 14, *Hymenostilbe lecaniicola.*

lides are subcylindric 7–10 × 2–2.5 μ terminating in acute apices. The conidia are fusoid, 2.5–3.4 × 1–1.5 μ and are catenulate.

Petch (12) states that in collections made by C. B. Williams in Trinidad and British Guiana synnemata of *Isaria pistillariaeformis* are associated with perithecial clavae of *Cordyceps tuberculata* (see no. 1).

HYMENOSTILBE Petch, The Naturalist **1931**: 101

Synnemata terete, subcylindric, often slightly attenuated upward, composed of longitudinal more or less parallel closely compacted hyphae (conidiophores); phialides in a hymenial layer covering the synnema, produced as terminal cells of short lateral branches from the longitudinal hyphae of the synnema or as lateral cells or buds directly from the longitudinal hyphae or less frequently as terminal cells of the longitudinal hyphae, subcylindric to clavate, narrowed above into short sterigmata or obtuse above with short or inconspicuous sterigmata, smooth or variously roughened in the upper portion; conidia one-celled, hyaline, smooth, single.

Type species: *Hymenostilbe muscarium* Petch.

Hymenostilbe differs from *Akanthomyces* principally in that its conidia are produced singly. Petch in his generic description states that the conidia are solitary or rarely catenulate. He (15) later, however, states that *Akanthomyces* is separated by the catenulate character.

8. HYMENOSTILBE DIPTERIGENA Petch. FIGS. 12–13; 23A–C
Trans. British Mycol. Soc. **16**: 212. 1932

Hosts attached to the substratum by dark brown mycelium which sometimes covers the insect; synnemata arising from the posterior part of the abdomen and between the segments of the body, subcylindric, somewhat attenuated upward, 4–12 mm. long, 0.2–0.5 mm. thick, composed of longitudinal somewhat interwoven, closely compacted hyphae the outer sometimes brownish and the inner hyaline; phialides forming a dense palisade layer covering the synnema, developed as terminal cells of short lateral branches from the longitudinal hyphae, cylindric to clavate, 14–28 × 3–4 μ, bearing scattered short cylindric to slightly capitate projections specially towards the apices, with sterigmata inconspicuous; conidia obovoid, 4–9 × 2–4 μ, hyaline, single.

North Carolina: On flies, Cranberry, Aug. 1887, R. Thaxter, Rel. Farl. 612 (M).

Tennessee: On flies, Burbank, Aug. 1887. R. Thaxter (FH 6192).

Nicaragua: On flies, El Recreo, November, 1945, Arthur G. Kevorkian No. 52 (FH and M).

In addition Petch (12) reported *H. dipterigena* as occurring on specimens from Panama, British Guiana, Tobago, New Guinea and Ceylon. The phialides of this species bear pronounced cylindric projections (FIGS. 23A & B). *H. dipterigena* is the conidial stage of *Cordyceps dipterigena* Berk. & Br. (12). Synnemata of *H. dipterigena* occur associated with perithecia clavae in Rel. Farl. 612 and FH 6192 (FIG. 12).

9. **Hymenostilbe lecaniicola** (Jaap) comb. nov. FIGS. 14; 24A–B

Isaria lecaniicola Jaap, Verh. Bot. Ver. Prov. Brandenburg **50**: 49. 1909.

Hirsutella lecaniicola (Jaap) Petch, Trans. British Mycol. Soc. **18**: 53. 1933.

Slight gray mycelia covering the hosts; synnemata several from an insect, cylindric to slightly clavate, up to 3 mm. long, 0.1–0.5 mm. thick, gray to brownish, pruinose, consisting of closely compacted, longitudinal, parallel hyphae; phialides arising laterally from the outer longitudinal hyphae or from the mycelium covering the host, scattered to moderately crowded, very variable, subcylindric, frequently with one or more constrictions giving a nodulose appearance, 10–30 × 4–5 μ, gradually attenuated at the apex into short acute to acuminate sterigmata 8–14 μ long, sometimes with several sterigma-like projections; conidia ellipsoid to broadly fusoid, 4–8 × 2–2.5 μ, hyaline single.

Michigan: On scale insects, Au Train Falls, Forest Lake, June 17, 1933, E. B. & E. E. Mains, 33–368 (M); Chatham, June 17, 1933, E. B. & E. E. Mains, 33–365 (M).

Quebec: On scale insects, Duchesnay, Aug. 24, 1938, E. B. Mains, 4227 (M).

Miss Charles (1) reports the species from the District of Columbia, Florida, Maryland, New York, Vermont and Virginia.

Hymenostilbe lecaniicola is the conidial stage of *Cordyceps clavulata* (Schw.) Ell. & Ev. (**13**). In the collections listed above the synnemata are old and the fungus had developed many perithecial clavae. The description has been mostly prepared from the specimen of Jaap Fungi Selecti Exsiccati No. 298 in the Bureau of Plant Industry, which is part of the type collection (**4**).

The phialides do not form as compact a layer as in most species of Hymenostilbe. Whether the several projections from the upper portion of some of the phialides are all sterigmata is uncertain. Conidia have not been seen attached to other than the terminal sterigma. The irregular somewhat nodulose appearance of some of the phialides is unusual (FIG. 24A).

The somewhat scattered distribution of the phialides is a condition which occurs more commonly in *Hirsutella* than in *Hymenostilbe.* Neither Jaap (**4**) or Petch (**13**) describes a mucous covering of the conidia and in the specimens which have been available for study such a covering has not been observed. Since the conidia of *Hirsutella* have a thick covering of mucus it seems best to place this species in *Hymenostilbe.*

10. HYMENOSTILBE APHIDIS Petch, Trans. British Mycol. Soc. **25**: 259. 1942

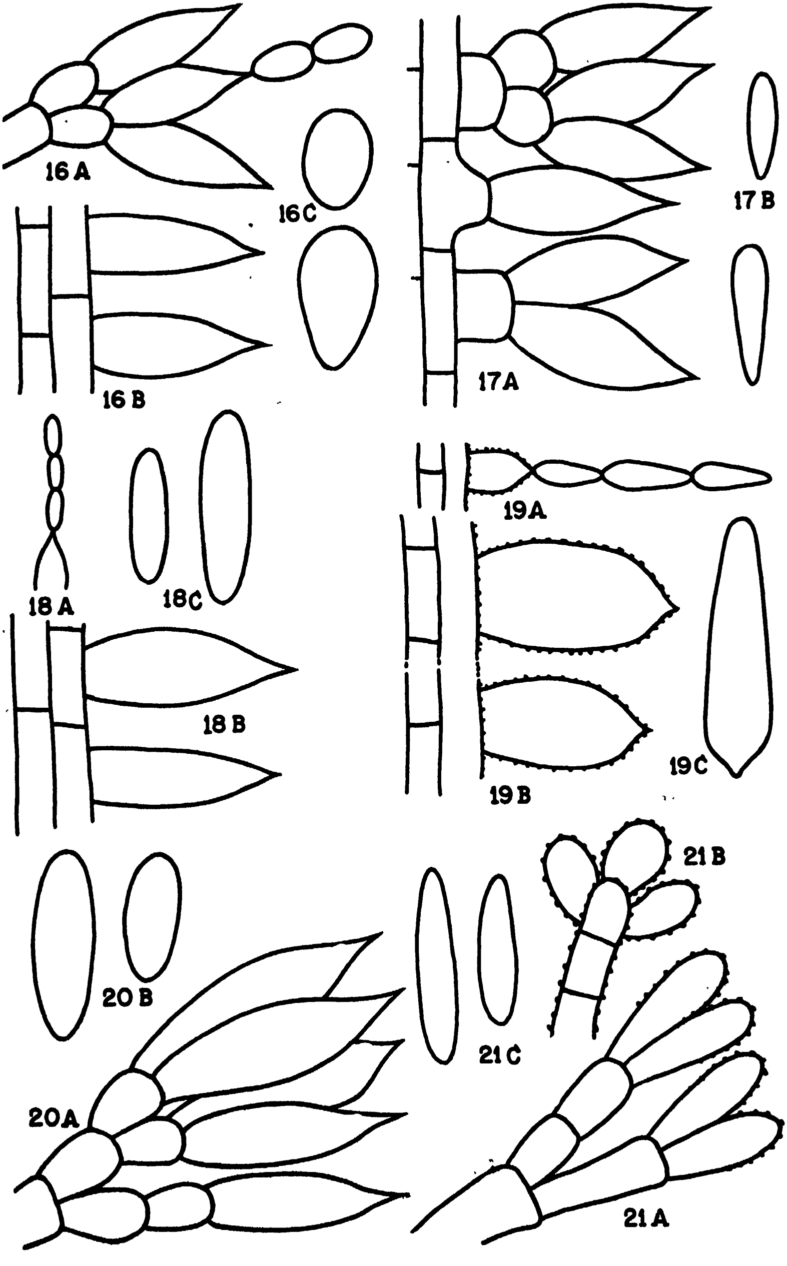
Synnemata solitary, rufous brown, up to 2 mm. long, 0.1 mm. thick, erect or suberect, straight or flexuous, cylindric or slightly thickened upwards, minutely pruinose; phialides forming a palisade layer over the synnema, conoid, narrowly flask-shaped or subcylindric, 12–18 × 4–6 μ, pale brown, abruptly narrowed at the apex into a short cylindric sterigma, 3–6 × 1 μ; conidia smooth, narrowly ovoid or fusoid, 9–15 × 4–5 μ, with a short truncate apeculus.

Dominica, B. W. I.: On aphids, January, 1940, R. G. Fennah.

Specimens of this species have not been seen. The description is adapted from that published by Petch who states that hyphae of *Hirsutella aphidis* Petch also occurred with the *Hymenostilbe.*

11. HYMENOSTILBE FORMICARUM Petch, Trans. British Mycol. Soc. **16**: 218. 1932

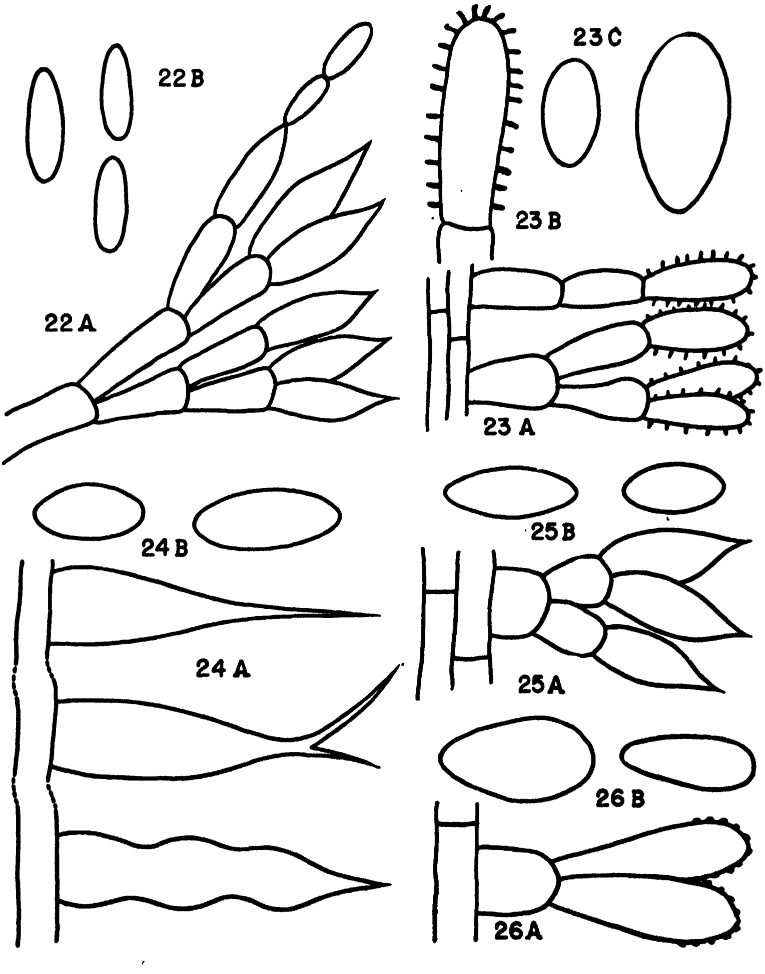
Synnemata simple, terete, up to 14 mm. long, 0.2 mm. thick below, enlarged to 0.3 mm. above, or furcate above, pale brown,



FIGS. 16–21. Phialides and spores of *Akanthomyces* and *Insecticola.*

ashy above, fibrillose below, pruinose above; phialides cylindric, 24 × 4 μ, verrucose above, with one or two broad truncate sterigmata; conidia narrowly clavate or subcylindric, 6–11 × 1–1.2 μ, one end acute, the other truncate or rounded.

Miss Charles (1) reports this species from Panama. Specimens have not been seen and the description is adapted from Petch who reports it associated with the perithecial clavae of *Cordyceps lloydii* Fawcett in collections from British Guiana.



FIGS. 22–26. Phialides and spores of *Insecticola* and *Hymenostilbe*.

12. **Hymenostilbe camponoti** sp. nov. FIGS. 25A–B

Synnema cylindricum, 8 mm. longum, 0.3 mm. crassum, furcatum, griseobrunneum, ex longitudinalis hyphis compositum; phialides densum hymenium formantes, subcylindricae vel clavatae 6–10 × 3–3.5 μ, superne acutae, sterigmatibus 4 μ longis; conidia late fusoidea, 4–6 × 2 μ, hyalina.

Ex Camponoto sp., Crescent City, Florida, in Herb. Univ. Mich.

Synnema arising between the head and thorax of an ant, cylindric, 8 mm. long, 0.3 mm. thick below, furcate above into two short branches, grayish brown, composed of longitudinal, closely compacted hyphae; phialides in a dense layer covering the synnema, terminal on short lateral branches from the outer hyphae, subcylindric, 6–10 × 3–3.5 μ, narrowing above to an acute apex terminated by a sterigma up to 4 μ long; smooth; conidia broadly fusoid, 4–6 × 2 μ, acute at the ends, hyaline.

From an ant, *Camponotus* sp., Crescent City, Florida (M).

This specimen was among undetermined collections in the Farlow Herbarium. It differs from *Hymenostilbe formicarum* in having smooth phialides with acute apices and in the size and shape of the conidia. Petch (12) has reported *Hymenostilbe melanopus* on ants in South America. As has been discussed elsewhere (8) the fungus on ants is not *H. melanopus* which was described by Spegazzini (19) on beetles. The *Hymenostilbe* on ants which is connected with *Cordyceps australis* differs from *H. camponoti* in having ovoid phialides which are minutely verrucose at the apex and with a short truncate sterigmata. The conidia are described as fusoid, 7–9 × 1 μ, with a truncate base.

13. HYMENOSTILBE SPHECOPHILA (Ditm.) Petch sensu Petch, Trans. British Mycol. Soc. 21: 55. 1937

Isaria sphecophila Ditm. ex Fr., Sys. Mycol. 3: 275. 1832.

Synnema arising from the thorax of a wasp, terete, up to 3 cm. long, 0.5 mm. thick, composed of longitudinal, parallel hyphae; phialides forming a compact palisade layer, arising as lateral cells from the longitudinal hyphae of the synnema, subcylindric to clavate, 16–24 × 4–5 μ, acute at the apices; conidia fusoid, 6–12 × 2.5–3.5 μ.

Puerto Rico: On wasp, Ins. Exp. Sta. Rio Piedras, R. A. Toro (FH).

This apparently is the Puerto Rican specimen in the Farlow Herbarium, to which Petch (14) refers in his discussion of *Isaria sphecophila* Ditm. He determined it as "*Cordyceps sphecocephala* (immature)." It is doubtful whether it is Ditmar's fungus. Ditmar's illustration (2) suggests a *Hirsutella* as Speare (18) has pointed out. Petch (14) recognized the possibility but decided that Ditmar's name applied to a *Hymenostilbe* because only specimens of a *Hymenostilbe* on wasps were known to him. He states that it is the conidial stage of *Cordyceps sphecocephala.*

14. **Hymenostilbe verrucosa** sp. nov. FIGS. 15; 26A–B

Mycelia subflava, floccosa, hospitis contegentia; synnemata anguste cylindrica, 1–4 mm. longa, 0.2–0.4 mm. crassa, pallide brunnea, ex longitudinalis hyphis composita; phialides cylindricae vel clavatae, densum hymenium formantes, 12–18 × 2–3 μ, superne verrucosae vel tuberculosae; conidia obovoidea, 6–8 × 2–3.5 μ hyalina.

Ex araneis, Kittery Point, Maine, R. Thaxter (FH 6270B).

Mycelia yellowish-white, cottony, covering hosts and attaching them to the substratum; synnemata narrowly cylindric or slightly clavate, 1–4 mm. long, 0.2–0.4 mm. thick, pale brown, composed of longitudinal hyphae; phialides forming a compact hymenium covering the synnema, produced as the terminal cells of short branches from the longitudinal hyphae, cylindric or clavate, 12–18 × 2–3 μ, verrucose or tuberculate at the apices; conidia obovoid, 6–8 × 2–3.5 μ, hyaline.

Maine: On spiders, Kittery Point, R. Thaxter (FH 6270B).

This collection was determined by Petch as *Hymenostilbe arachnophila* (Ditm.) Petch. It agrees with the description given by him (11) for two specimens in the Kew Herbarium from Southampton, England. As has been discussed under *Akanthomyces aranearum,* the name *Isaria arachnophila* Ditm. cannot be applied with any certainty to the several species of fungi on spiders. It is more likely to be a species of *Akanthomyces* than *Hymenostilbe.*

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EXPLANATION OF FIGURES

FIG. 1. *Akanthomyces aculeata,* A.H.S. 26556. Development on adult moth showing scattered synnemata, × 3. FIGS. 2–3. *Akanthomyces angustispora,* type; FIG. 2. Synnemata developing from coleopterous larva, × 3. FIG. 3. Lateral hymenial surface of synnema showing crowded phialides, × 800. FIG. 4. *Akanthomyces ampullifera,* type. Synnemata developing from a gnat, × 6. FIGS. 5–6. *Akanthomyces aranearum,* FH 4123; FIG. 5. Synnemata from a small spider, × 2. FIG. 6. Lateral hymenial surface of synnema showing somewhat scattered phialides, × 800. FIGS. 7–8. *Insecticola clavata,* type; FIG. 7. Infected cricket showing scattered synnemata, × 3. FIG. 8. Synnemata, × 25. FIGS. 9–10. *Insecticola pistillariaeformis,* FH 2584; FIG. 9. Infected moth showing scattered synnemata, × 3. FIG. 10. Synnemata with upper sporogenous portions covered with pulverulent masses of conidia, × 25. FIG. 11. *Insecticola fragilis,* type, showing scattered synnemata, × 3. FIGS. 12–13. *Hymenostilbe dipterigena;* FIG. 12. Two synnemata (right) associated with three perithecial bearing clavae (left) of *Cordyceps dipterigena* on a fly, FH 6192, × 3. FIG. 13. Synnemata from a fly, Kervorkian 52, × 5. FIG. 14. *Hymenostilbe lecaniicola* showing synnemata arising from scale insects, Jaap Fungi Sel. Exsic. 298, × 5. FIG. 15. *Hymenostilbe verrucosa,* type, showing synnemata arising from small spider, × 6. FIGS. 16 A–C. *Akanthomyces aculeata:* A. Phialides and catenulate conidia terminating a longitudinal hypha of a synnema, × 2000; B. Phialides arising as lateral cells of an outer hypha of the synnema, × 2000; C, Conidia, × 3000. FIG. 17 A–B. *Akanthomyces angustispora:* A. Phialides arising as lateral cells or terminating short lateral branches covering the synnemata, × 2000; B, Conidia, × 3000. FIG. 18 A–C. *Akanthomyces ampullifera:* A. Chain of conidia, × 1000; B. Phialides arising as lateral cells from outer hypha of synnema, × 2000; C. Conidia, × 3000. FIG. 19 A–C. *Akanthomyces aranearum:* A. Phialide with catenulate conidia, × 1000; B. Phialides arising as lateral cells of outer hyphae of synnema, × 2000; C. Conidium, × 3000. FIG. 20 A–B. *Insecticola clavata:* A. Phialides terminating hyphal branches in the upper portion of the synnema, × 2000; B. Conidia, × 3000. FIG. 21 A–C. *Insecticola fragilis:* A. Phialides terminating hyphal branches in the upper portion of synnema, × 2000; B. Terminal portion of a short flexuous hypha from the stipe, × 2000; C. Conidia, × 3000. FIG. 22 A–B. *Insecticola pistillariaeformis:* A. Phialides and catenulate conidia terminating branches in the upper portion of the synnema, × 2000; B. Conidia, × 3000. FIG. 23 A–C. *Hymenostilbe dipterigena:* A. Phialides terminating lateral branches covering the synnema, × 1000; B. Phialides, × 2000; C. Conidia, × 3000. FIG. 24 A–B. *Hymenostilbe lecaniicola:* A. Phialides arising as lateral cells over the surface of the synnema, × 2000; B. Conidia, × 3000. FIG. 25 A–B. *Hymenostilbe camponoti:* A. Phialides terminating short lateral branches covering the synnema, × 2000; B. Conidia, × 3000. FIG. 26 A–B. *Hymenostilbe verrucosa:* A. Phialides from short lateral branches covering the synnema, × 2000; B. Conidia, × 3000.

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OBSERVATIONS ON KERATIN DIGESTION BY MICROSPORUM GYPSEUM[1]

ROBERT M. PAGE[2]

(WITH 2 FIGURES)

Since the dermatophytes occur in superficial tissues (hair, skin, nails) of mammals, and since the chief proteinaceous constituent of these tissues is keratin, it has often been assumed that the dermatophytes are capable of digesting keratin. This presumed ability is of interest, because under normal conditions, keratins are resistant to hydrolysis by such proteolytic enzymes as pepsin or trypsin. The reason for the indigestibility of keratins must lie in their properties. These scleroproteins, as is well known, are insoluble in water, dilute acids and bases, ethanol, or salt solutions. They are highly polymerized compounds made up of polypeptide chains bound laterally by disulfide linkages which maintain the three-dimensional shape of the molecule.

Dermatophytes have been grown in culture with various types of keratin as substrata, but in no case have the cultures been observed in sufficient detail to establish that keratin is actually digested. For example, wool (Bonar and Dreyer, **1932**; Rogers,

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[1] Portion of a thesis submitted to the faculty of Harvard University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The author wishes to express his gratitude to Prof. Wm. H. Weston for his encouragement and guidance during the course of this study and preparation of the manuscript. This investigation was carried out during the tenure of a Junior Research Fellowship, National Institute of Health.

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Hirschmann, and Humfeld, **1940**), human hair (Roberts, **1894**; Bonar and Dreyer, **1932**; Williams, **1934**, **1934a**, **1935**), and feathers (Macfadyen, **1894**; Tate, **1929**) have supported the growth of a variety of fungi belonging to this group. Growth on substrata as complex as hair, feathers, or wool cannot, however, be considered proof of the ability of these fungi to digest keratin.

A detailed study of the growth of dermatophytes on keratin-containing substrata was of interest, therefore, to establish the ability of these fungi to digest keratin, to consider the process with respect to general problems of the relation of organisms to insoluble substrata, and to compare the features of the growth *in vitro* with those encountered in the clinic. While these objectives of this investigation were not fully attained, such progress as has been made is presented in the hope that it may be of service to others working in the same field.

MATERIALS AND METHODS

Cows' horn, fingernails, wool, and human hair were selected as representative types of keratinized tissue and were prepared for study in the following ways. Cows' horn, obtained from a slaughter house, was reduced to a finely particulate state in two ways. In some cases a file was used, and, though tedious, this method was advantageous in that the particles were thin ribbons with a large surface area. In other cases, the horn was broken up and ground in a laboratory model Wiley mill to pass a 40 mesh screen. Both methods were designed to avoid the alteration of the properties of keratin which occurs when it is subjected to prolonged grinding in a ball mill (Routh, **1940**). For some observations the ground horn was incorporated in 2.0% tap water agar and sterilized by autoclaving at 15 lbs. pressure for 15 minutes. In a second series of observations heat sterilization was avoided by the use of chloroform, and the horn was added to autoclaved 2.0% agar which had cooled almost to the point of gelation. The medium was agitated while it was poured into Petri dishes, so that the particles were distributed and partially suspended in the agar. The fingernails were prepared by grinding in the Wiley mill, after which they were washed with 0.1% sodium taurocholate and further cleaned by extraction with ether and acetone. The powder was added to 2.0% agar and sterilized by autoclaving. The wool, which was unscoured and solvent-extracted, was combed to remove debris, washed with soap and water, extracted repeatedly with ether, and dried. Following sterilization with 70% ethanol, it was placed in a sterile Petri dish and warm agar was added. The pigment in human hair obscured details of the fungus attack; therefore, grey hairs which were devoid of pigment were selected for study. The hair was prepared by washing it with 0.1% sodium taurocholate and rinsing with water and acetone. It was then extracted repeatedly with small portions of ether to remove any fats remaining and sterilized by immersing it in 70% ethanol for one hour. After sterilization a few strands were placed in a sterile Petri dish and warm agar was added.

The media were inoculated, and the cultures were incubated at 30° C. The cultures were observed frequently through the bottom of the inverted Petri dish with the 16 mm. objective of the microscope. Typical areas of the medium were selected, and photomicrographs of these areas were taken at suitable intervals by means of a Leitz Ibso attachment.

In addition to the observations on human hair embedded in agar, details of the attack on human hair were studied by placing hairs in Petri dishes with sterile tap water and inoculating with *Microsporum gypseum.* Hairs were removed from the cultures at various intervals after inoculation, mounted in lactophenol with acid fuchsin, and observed with the microscope.

OBSERVATIONS

Horn. In preliminary experiments several dermatophytes were grown on the horn medium. Plates of autoclaved horn medium were inoculated with three isolates each of *Microsporum gypseum, M. canis, M. Audouini, Trichophyton mentagrophytes, T. rubrum, T. tonsurans,* and *Epidermophyton floccosum.*[3] All of the fungi grew on the horn medium, but growth was diffuse and prostrate when compared with that on Sabouraud's agar. After two weeks, clear zones were formed under the centers of the colonies because of the partial disappearance of the horn particles from the medium. As a quantitative measure of the ability of the fungi to digest horn, the diameter of the clear zones did not prove satisfactory, because the margins of the zones were difficult to delimit, and the degree of transparency of the zones varied with the different strains. *M. gypseum, M. canis,* and *T. rubrum* produced zones which were larger with respect to the diameter of the colony and more transparent than the zones produced by the other fungi. *M. Audouini,* on the other hand, formed relatively small zones in which the particles of horn were translucent rather than transparent. The size and transparency of the cleared zones produced by one isolate of *M. gypseum* (S–159) suggested that it was the most active of the fungi tested in its ability to digest horn. This strain also grew rapidly and vigorously, and was therefore selected for use in the observations which follow.

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[3] For these cultures, the author is indebted to Miss Margarita Silva of the School of Tropical Medicine, San Juan, Puerto Rico and to Dr. C. W. Emmons of the National Institute of Health, Bethesda, Md.

In a series of detailed experiments with *Microsporum gypseum* (Bodin) Guiart and Grigorakis microscopic observations on individual fragments of horn showed that they became translucent and ultimately disappeared almost completely. A series of photomicrographs showing the digestion of a fragment of horn which had been ground in the Wiley mill and partially sterilized with chloroform is shown (FIG. 1). Although the chloroform did not effect complete sterilization of the horn, no contaminants appeared in the field selected for study. Since the fragment was located at some distance from the site of inoculation, the hyphae did not reach the vicinity of the particle until between 24 and 48 hours after the plate had been inoculated. The first hyphae to enter the field of observation were hyphae which radiated from the site of inoculation, and the direction of their growth appeared to be little influenced by the presence of the horn particles. After these hyphae had grown past a particle, they gave rise to numerous side branches which grew toward the particle and became closely applied to it (FIG. 1, b). Very little change in the appearance of the particle was apparent at this stage. By 96 hours after inoculation of the plate, however, a definite alteration of the appearance of the particle was visible (FIG. 1, c). The margin of the particle had

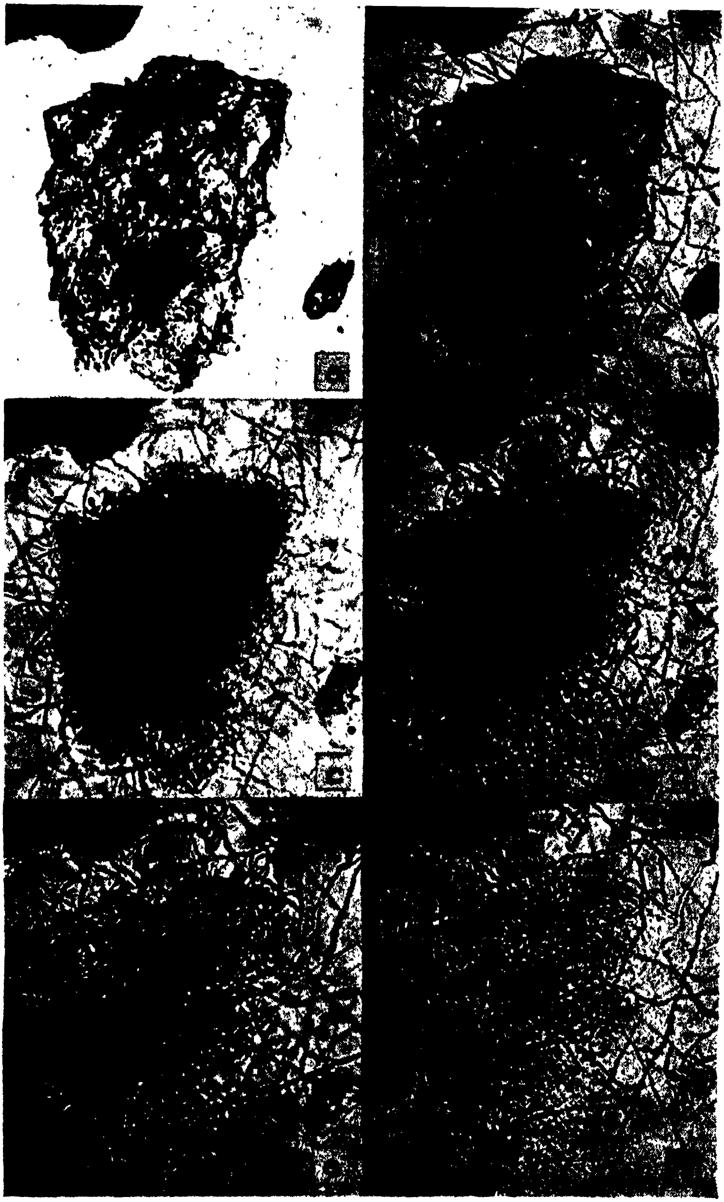

FIG. 1. Digestion of cows' horn by *Microsporum gypseum.*

become indistinct in outline and somewhat translucent. The zone of translucence gradually extended until the particle had all but disappeared at the end of 240 hours after inoculation.

While the sequence of events described in the preceding paragraph is typical, the time required for the digestion of a fragment varied considerably depending on its size, shape, proximity to the point of inoculation, and previous treatment. The thin ribbons of horn produced by filing and sterilized by autoclaving disappeared more rapidly than the thicker particles produced by the Wiley mill and sterilized with chloroform.

Fingernails. Preliminary observations on the digestion of fragments of human fingernails indicated that the process was very similar to the digestion of horn. The particles of fingernail in microscopic appearance closely resembled those of horn, they were approached by the hyphae in the same way, and they were digested in approximately the same length of time.

Wool. Wool represents a more complex form of keratinized tissue than does horn or fingernails, because the molecular units are oriented longitudinally, and each fiber is differentiated into a central region, the cortex, composed of spindle-shaped cells and an external sheath, the cuticle, made up of thin, overlapping scales. The digestion of wool was not studied in detail, because such studies are currently in progress in another laboratory; however, a brief description is included for the sake of completeness.

In one series, observations on the digestion of a wool fiber were made daily over a 10-day period. The fungus appeared to be able to penetrate the cuticle readily, since many of the fibers were attacked at several points along their length. Whether the hyphae gained entry to the cortex through or between the scales of the cuticle was not determined, but in any case, there was little distortion of the fiber at the point of penetration. After penetrating the cuticle, the hyphae grew longitudinally within the cortex. Accompanying the longitudinal growth were opaque areas which appeared as dark shadows. These opaque areas extended, and in some cases the longitudinally oriented hyphae could be distinguished within them. The substance of the cortex gradually disappeared, so that by a late stage little remained of the fiber but the cuticle which

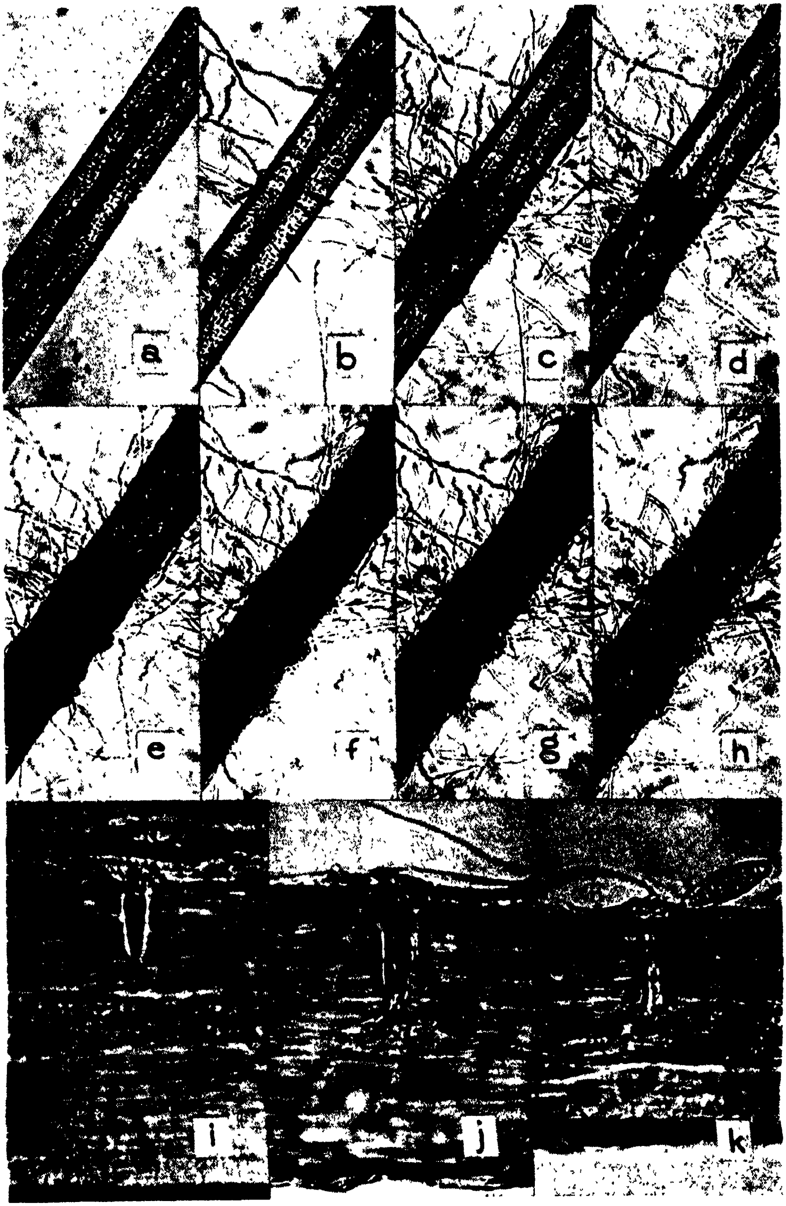

FIG. 2. Human hair attacked by *Microsporum gypseum*.

enclosed the hyphae. The cuticle appeared to be more resistant to the action of the fungus than the cortex, and it was observed to be completely digested in only limited areas.

Human hair. Human hairs are more complex histologically than wool, since they are differentiated into three regions. In addition to the cortex and the cuticle, there is a third region, the medulla, which occupies the central axis of the hair shaft and may be continuous, fragmental, or absent in any one hair. The medulla, when present, is composed of somewhat rounded cells.

A series of photographs showing a hair under attack by *M. gypseum* is shown (FIG. 2, a–h). At the site chosen for study the hair was approximately 115 μ in diameter, and it possessed a continuous medulla. Forty-eight hours after the plate had been inoculated the first hyphae entered the field and gave rise to branches which approached the hair shaft. In some cases the hyphae proceeded along the surface of the hair for some distance. These hyphae were not visible in unstained preparations but could be observed easily in hairs fixed and stained with acid fuchsin in lactophenol. These hyphae which were closely appressed to the hair shaft appeared to be the ones responsible for penetration. The mode of penetration was difficult to establish: in one case a scale of the cuticle appeared to be loosened by mechanical action, but it seems possible that penetration is enzymatic.

Two processes took place almost concurrently after penetration had been effected. First, there was a proliferation of the fungus beneath the cuticle together with an apparent swelling of the cortex, so that a vesicle or eruption was formed. The size of these eruptions varied considerably; sometimes they were relatively small (FIG. 2, i–k), while occasionally they attained a height equal to the diameter of the hair. Second, some of the hyphae digested their way directly into the cortex of the hair to form intrusions which were first conical (FIG. 2, i) and later became cylindrical (FIG. 2, j, k). These intrusions were best studied in hairs fixed and stained with acid fuchsin in lactophenol. The unattacked portions of the hair remained unstained, but the intrusions were deeply stained by the acid fuchsin. In medullated hair the intrusions usually penetrated only as far as the medulla, but occasionally they appeared to pass through the hair shaft. Breakdown of the cortex appeared to extend longitudinally from the intrusions (FIG. 2, j). The attacked areas of the cortex appeared as dark shadows which gradually extended until the entire hair had lost its original appearance (FIG. 2, h).

Human hair under attack by *M. gypseum* was studied with the polarizing microscope and compared with hair viewed with ordinary light. Normal hair showed strong birefringence when viewed with polarized light. The intrusions, however, showed no birefringence and remained dark when observed through crossed Nichol's prisms in strong contrast to the unattacked portions of the hair. Regions of the hair where the cortex had been attacked showed diminished birefringence at the edges of the zone of attack and no birefringence where the shadows appeared darkest under ordinary light.

DISCUSSION

From a theoretical standpoint, the most significant result of the present investigation was the indication that *Microsporum gypseum* and other dermatophytes are capable of digesting keratin. The almost complete disappearance of particles of horn and fingernails and the loss of birefringence by hair during attack by the fungus indicate that keratin was digested.

Observational evidence for keratin digestion, while not quantitative, is superior in several respects to other possible criteria and is, therefore, a valuable adjunct to them. The criterion of the measurement of the tensile strength of fabric which has been widely used in the study of the degradation of cellulosic textiles is open to objection when applied to multicellular keratinized material, because a decrease in tensile strength might indicate a breakdown of the intercellular cement rather than of the keratinized cells. Similarly, growth of an organism on keratin-containing substrata or the accumulation of by-products of metabolism in a medium might prove misleading unless supported by other evidence.

While keratins are resistant to the action of proteolytic enzymes of the higher vertebrates (*cf.* Routh and Lewis, **1938**), digestion of keratins has been demonstrated with several organisms, notably the clothes moth (Linderstrøm-Lang and Duspiva, 1935), the goshawk and vulture (Stanković, Arnovljević and Matavulj, 1929), and certain Actinomycetes (Jensen, 1930). Moreover, it seems probable that some of the Chytrids are able to digest keratins, since they appear to be limited in growth to keratinized tissues (Karling, 1948).

Although contact between hyphae and substratum is not a requisite for digestion, the hyphae must be close to a fragment before it is digested. Even with horn sterilized by autoclaving, the particles showed no sign of digestion until the hyphae had been in the near vicinity of a particle for at least 24 hours. This observation is in agreement with the macroscopic appearance of plates of horn medium, for in no case was the clear zone in the medium observed to extend beyond the margin of the colony. For example, on the horn medium the diameter of 15-day-old colonies of *M. gypseum* averaged 75 mm., while the clear zones averaged only 53 mm. in diameter. This result was in contrast to that obtained on a medium of coagulated casein where the clear zone extended beyond the hyphal tips. These results suggest either that the enzyme responsible for the digestion of keratin does not diffuse rapidly, or that the digestion of the large particles of horn was so slow that the hyphae grew past the fragments before any change in their appearance could be detected. Another possibility, of course, is that the enzyme is oxidized or otherwise inactivated after it has diffused some distance from a hypha, but observations provide no clue as to which of these alternative explanations is valid.

Actual contact of the hyphae with the particle of horn is unnecessary for digestion to occur, because the horn disappeared from within the meshes of the reticulum of branch hyphae which was formed around a fragment of horn. This behavior suggests, therefore, that the enzyme responsible for the digestion of the keratin is not adsorbed on the surface of the hyphae.

With horn there was no evidence of orientation of the hyphae with respect to the orientation of the micelles of the substratum. With wool, the hyphae ran parallel to the orientation of the micelles, but the direction may have been the result of the physical confinement imposed by the cuticle. Hyphae attacking human hair first ramified over the surface, then penetrated the hair at right angles to the axis, and finally extended into the cortex in a direction parallel to the axis. *Microsporum gypseum,* in its attack on keratin, did not, therefore, show the precise orientation with respect to the direction of the micelles which is characteristic of the attack of certain organisms on cellulose (Stanier, **1942**; Bailey and Vestal, **1937**).

From the point of view of medical mycology, the ability of *M. gypseum* to attack fingernails vigorously *in vitro* is of interest, since this organism does not appear to be an etiologic agent of onychomycosis. Some factor other than the nature of the keratin must be responsible for the distribution of the fungus on the host. The growth on hair also differs from that observed in the clinic. The spore sheath, which characteristically surrounds the hair shaft in cases of tinea capitis caused by members of the genus *Microsporum,* was absent in the hairs embedded in agar, and both macroconidia (*fuseaux*) and microconidia were produced in abundance on the surface of the medium. Invasion of the hair shaft occurred regularly in these cultures, whereas in cases of tinea capitis caused by *M. gypseum,* the reports are conflicting. Carrión and Silva (**1944**) report that invasion occurs, while Lewis and Hopper (**1943**) state that it does not.

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EXPLANATION OF FIGURES

FIG. 1. Photomicrographs showing representative stages in the digestion of a particle of cows' horn by *Microsporum gypseum*. a, Appearance of particle at time of inoculation. b–f, Appearance of particle after 52, 96, 120, 144, and 196 hours respectively. × 112.

FIG. 2. Photomicrographs showing human hairs attacked by *Microsporum gypseum*. a–h, Successive stages in the attack of *M. gypseum* on a human hair embedded in agar. × 75. a, Hair at time of inoculation. b–h, Photographs taken daily from the second to the eighth day respectively. i–k, Hairs fixed and stained with lactophenol and acid fuchsin. × 425. i, Portion of hair showing eruption of cuticle and intrusion of fungus into cortex. j, Portion of non-medullated hair showing initiation of longitudinal attack. k, Intrusion penetrating to medulla. Note macroconidium of *M. gypseum* adhering to surface of hair.

ASHBYA GOSSYPII—ITS SIGNIFICANCE IN NATURE AND IN THE LABORATORY[1, 3]

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(WITH 4 FIGURES)

For the past several years investigations concerning the yeast-like fungus *Ashbya gossypii* (Ashby and Nowell) Guilliermond have centered around its capacity to synthesize large amounts of riboflavin, or vitamin B_2. Aside from its flavinogenic potentialities, however, *A. gossypii* possesses certain other characteristics which accentuate its importance to man. Its pathogenicity towards important crop-plants is of considerable economic concern. Its physiological activities are utilized in certain fundamental studies and in a microbiological assay for the vitamin biotin. Finally, its anomalous morphology and cultural behavior present interesting problems relative to its classification and possible phylogenetic relationships. The present report is an attempt to assemble available information concerning these unique characteristics of *A. gossypii* and its potentialities as a vitamin producer.

OCCURRENCE AND PATHOGENICITY

Ashbya gossypii (Ashby and Nowell) Guilliermond, otherwise known as *Nematospora gossypii* Ashby and Nowell, or *Ashbia gossypii* Ciferri and Fragoso (Ashby and Nowell, **1926**; Guilliermond, **1927**, **1928**; Fragoso and Ciferri, **1928**; Ramsbottom, **1942**)

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[1] Report of a study made under the Research and Marketing Act of 1946.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[3] A portion of the material here reported was included in a thesis submitted by the senior author to the faculty of the University of Illinois, June 1949, in partial fulfillment of the requirements for the degree of doctor of philosophy in bacteriology.

was possibly first observed by Bartlett in 1907. He noted the characteristic spores of either this organism or *Nematospora coryli* Peglion in infected cotton bolls from British Guiana, but was unable to isolate the organism in pure culture. The first isolation and characterization was recorded by Nowell in 1916–1917 who found the organism to be one of four species infecting cotton in the British West Indies (Nowell, **1915**, **1916**, **1917**, **1917a**). During the same period Nowell (**1917a**, **1918**) obtained further evidence regarding its occurrence. He observed that certain sucking insects were of importance in its dissemination and that tomatoes could also serve as a host. The organism was more fully characterized and given the name *Nematospora gossypii* some years later when it was isolated from the lint and seeds of cotton originating in the British West Indies and Nyasaland, and from the seeds of jimson weed and milkweed (Ashby and Nowell, **1926**).

It has since been reported as a widely distributed and destructive pathogen of a variety of important economic crop-plants, including cotton, coffee, citrus fruits, tomatoes, okra, and various legumes (table 1). It has been isolated in North America only occasionally.

Other possible hosts have been reported by Wingard (**1925**), and the list could undoubtedly be expanded were the host plants of the insects responsible for the transmission of this and related microorganisms thoroughly investigated.

In cotton, the disease produced through infection with *Ashbya gossypii* and related organisms in the genera *Nematospora, Spermophthora,* and *Eremothecium* is variously termed "Stigmatomycosis," "Internal Boll Rot," or "Cotton Staining." The organisms infect the developing lint fibers which become dirty-yellowish in color and mat onto the seeds. The seed coats become stained brown in spots and as the bolls age the locks become totally retarded and the lint is reduced to a papery membrane. The seeds are not penetrated unless mechanical injury occurs. Infection results either in premature dropping of the bolls, or in a drying out of those which remain on the plant. The characteristic symptoms of the disease have been more fully described elsewhere (Pearson, **1934a**, **1947**; Wallace, **1939**; Wickens, **1947**). Tremendous annual losses occur in African cotton-growing regions as a result of this infection.

In coffee, the seeds are affected, the disease appears in the form of a dry rot with blackening and shrinking of the beans. This leads to "floats" after pulping, and to cracking of the beans in the drying frames (Wallace, 1931, 1932, 1939). When infections occur in

TABLE I

REPORTED HOSTS AND GEOGRAPHIC LOCATION OF *Ashbya gossypii* ISOLATIONS

Reported host	Location
Asclepias curassavica	Trinidad, B.W.I. (1)*
Centrosema plumieri	Belgian Congo (16)
Citrus aurantium	Florida (17)
Citrus nobilis	Florida (17)
Coffea arabica	Belgian Congo (8), Kenya (7, 9), Tanganyika (16)
Coffea robusta	Uganda (16)
Datura metel	Montserrat, B.W.I. (1)
Glycine max	Belgian Congo (16), Union of S. Africa (16)
Glycine soja	Belgian Congo (16), Union of S. Africa (16)
Gossypium spp	Anglo-Egyptian Sudan (19), Antigua, B.W.L. (1, 11), Bahama Is. (19), Belgian Congo (2, 12, 14, 18, 20), Bequia, B.W.I. (11), Brazil (16), British Guiana (11), Burma (12), Dominican Rep. (3), Grenadine Is., B.W.I. (1), Fiji (19). Jamaica (19), Kenya (19), Montserrat, B.W.I, (1, 11), Natal (19), Nevis, B.W.I. (1, 11), N. Rhodesia (19), Nyasaland (1, 13, 19), Portugese E. Africa (19), Queensland (19), S. Nigeria (6, 19), S. Rhodesia (5, 19), St. Vincent, B.W.I. (1, 11, 19), Swaziland (19), Tanganyika (16, 19), Trinidad, B.W.I. (1), Uganda (4, 19), Union of S. Africa (10, 15, 19)
Hibiscus sp	Brazil (16)
Hibiscus cannabinus	Uganda (16)
Hibiscus esculentus	Uganda (16)
Lycopersicum esculentum	St. Vincent, B.W.I. (11)
Persea gratissima	Uganda (14)
Phaseolus lunatus	Belgian Congo (16)
Phaseolus mungo	Union of S. Africa (16)
Phaseolus vulgaris	Union of S. Africa (16)
Sida sp	Brazil (16)
Sterculia platinifolia	Union of S. Africa (16)
Vigna sp	Belgian Congo (16)

* References refer to citations in table: (1) Ashby and Nowell, 1926; (2) Brixhe, 1938; (3) Fragoso and Ciferri, 1928; (4) Hansford, 1930; (5) Hopkins, 1932; (6) Laycock, 1935; (7) LePelley, 1942; (8) Leroy and Hendrickx, 1941; (9) McDonald, 1932, 1933; (10) Moor, 1930; (11) Nowell, 1915. 1916, 1917, 1917a, 1923, 1939; (12) Rhind, 1927; (13) Richardson, 1941; (14) Steyaert, 1934, 1934a, 1936, 1939, 1946; (15) Ullyett, 1930; (16) Wallace, 1932, 1939; (17) Weber, 1933; (18) Publ. Inst. nat. Etud. agric. Congo belge, 1939, 1941, 1943; (19) Emp. Cott. Grwg. Repts., 1926–1948; (20) Bull. Agric. Congo belge, 1941.

leguminous plants, the disease is generally termed "Yeast Spot." The seeds become dry, shrivelled, and dark (Wallace, **1932**).

Numerous studies sponsored primarily by the Empire Cotton Growing Corporation of England have demonstrated conclusively that *Ashbya gossypii* and related micro-organisms are transmitted from plant to plant by certain hemipterous insects (see Emp. Cott. Grwg. Corp. Repts., **1926–1948**; Bull. Agric. Congo Belgé, **1941**; Frazer, **1944**; Hansford, **1927**, **1930**; Laycock, **1935**; LePelley, **1942**; Marsh, **1926**; McDonald, **1932**, **1933**; Moor, **1930**; Nowell, **1917a**; Pearson, **1934**; Rhind, **1927**; Steyaert, **1939**, **1946**; Ullyett, **1930**; Wallace, **1932**; Weber, **1933**; Wickens, **1942**; Williams, **1934**). Various species of *Dysdercus* (cotton stainers) have been the most frequently observed carriers, with species of *Nezara* (green bug), *Leptoglossus* (leaf-footed tomato bug), *Phthia* (red tomato bug), *Antestia* (coffee bug), and *Callidea* less often implicated. *Antestia* species have been observed as carriers in all cases of coffee bean infection.

These insects mechanically transmit the organisms from plant to plant during the course of their feeding. The spores or mycelium are carried as external contaminants on the mouth parts and in the deep stylet pouches. Spores apparently do not remain viable or germinate after reaching the intestinal tracts of the insects, but may germinate in the stylet pouches. Spreading of the organisms may be accomplished in two ways. In some cases, spores or mycelia are cast off with the exuviae during moulting and may, by chance, enter an open wound in the plant, the fungus itself being unable to penetrate the outer cells of the host. The most important mode of infection is through injection of the spores by the insects into the tissue of the host. The characteristic needle-shaped spores of *A. gossypii* and related organisms are especially well adapted for this mode of infection. In experimental trials, spraying or surface inoculation of susceptible hosts are usually unsuccessful, while injection by syringe usually results in active infection.

A number of factors affect the degree of infection of cotton bolls, including age and sugar content of the bolls, and humidity. Young bolls are highly susceptible, whereas bolls four to five weeks or older are relatively resistant to infection; the sugar content of bolls ranges from approximately 50 per cent at one week to less than 1 per cent at maturity, accompanied by a steady decline in susceptibility; high humidity increases the incidence of infection (Marsh, **1926**; Emp. Cotto. Grwg. Repts., **1926–1948**; Hopkins, **1932**; Pearson, **1934**, **1939**, **1947**; Steyaert, **1936**, **1938**, **1939**; Rainey, **1940**, **1948**; Wickens, **1947**).

The staining of cotton by these organisms is due apparently to a modification of the protoplasm of the central canal of the lint hairs (Marsh, **1925**). Pearson (**1934**) enlarged upon this theory by indicating that such a modification followed killing of the fibers by a toxin secreted by the organisms. He reported that decrease in susceptibility with age of the bolls was due to evacuolation of the lint hairs and drying which impeded the movement of toxic substances within the hairs. In later experiments Pearson (**1947**) was unable to redemonstrate toxin production by *A. gossypii.*

The fact that *A. gossypii* is an important plant pathogen has caused some concern regarding its use in an industrial process for the production of riboflavin. However, our attempts to isolate viable cultures from drum- or spray-dried fermentation residues have been unsuccessful. In addition, preliminary thermal-death-time studies have indicated that the organism is rather susceptible to moderate temperatures under laboratory conditions, being killed in less than 20 minutes at 60° C and in less than 5 minutes at 70° C when exposed in fermented liquors.

TAXONOMY AND RELATIONSHIPS

Ashbya gossypii offers a challenge to the taxonomist, since it appears to possess characteristics of both phycomycetous and ascomycetous fungi. Its anomalous morphology presents problems in classification and poses questions as to its phylogenetic relationships.

Nowell (**1917**) first pointed out the close relationship of this organism to certain species of *Nematospora, Eremothecium,* and what is now the genus *Spermophthora.* He indicated that not until spore formation occurred could these three genera be separated. *Ashbya gossypii* most closely resembled *Nematospora* in the mode of formation and morphology of its spores, but differed in its strict adherence to a mycelial habit whereas species of *Nematospora* grew primarily as yeast-like forms. He regarded the sporiferous sacs of these organisms as sporangia and for that reason felt that the genus *Nematospora* should not be included among the Saccharomycetes. At this time he designated *A. gossypii* simply as species "C." Wingard (**1925**) further noted the relationships between species C and various *Nematospora* species but, following cytological studies of *N. phaseoli,* he advanced the opinion that the sporiferous sacs were, in fact, asci, and that these organisms were Saccharomycetes. He pointed out that species C appeared to have characteristics in common with such genera as *Nematospora, Monospora, Coccidiascus, Eremothecium, Protascus,* and certain species of *Endomyces.* Ashby and Nowell (**1926**) later characterized and named species C *Nematospora gossypii,* based on the close conformity of its sporiferous sacs to those of other species of *Nematospora,* and on the specialized spores. However, the usual mycelial habit of *A. gossypii* was in direct contradiction to the yeast-like habit of *Nematospora* species generally. They doubted that the organism was a Saccharomycete and left open the question of the exact nature of its sporiferous sacs. They pointed out the common salient features of the three genera, *Spermophthora, Nematospora,* and *Eremothecium.*

In a series of papers devoted to the cytology and phylogeny of the lower Ascomycetes, Guilliermond (**1927, 1928, 1935, 1936**) suggested placing the organism in the *Hemiascomycetes* but assigned to it a new genus name, *Ashbya.* This suggestion was based on his observations of spore formation at the expense of a sporoplasm, the presence of an epiplasm, even numbers of spores, and two mitoses of the nuclei prior to spore formation. He considered the fruiting body of *A. gossypii* as intermediate between a sporangium and an ascus because of its multinucleate nature, and pointed out that the organism was related to such genera as *Dipodascus, Protomyces, Endogone, Taphridium, Spermophthora,* and *Eremothecium.*

Fragoso and Ciferri (**1928**) increased the synonomy still further by presenting a Latin description of the organism under the generic name *Ashbia.* They considered the organism to be a Saccharomycete.

Stelling-Dekker (1931) omitted the genus *Ashbya* from her classification of the sporogenous yeasts, and considered its position doubtful, representing no more than a section of the genus *Nematospora* in the subfamily Nematosporoideae and family Endomycetaceae. According to her treatment, it would be related to the genera *Monosporella* and *Coccidiascus.*

Dodge (1935) retained the generic name *Ashbya,* but set up a separate family, Ashbyaceae, under the order Endomycetales to include this genus, as well as the genera *Piedraia* and *Eremothecium.*

It would thus appear that taxonomists generally agree upon placing the organism in the order Endomycetales of the Hemiascomycetes. From this point on, further attempts to classify *Ashbya gossypii* differ. It appears to possess characteristics of several families. If one accepts Martin's system of classification (1948), *A. gossypii* is seen to possess characteristics which suggest relationships with three different families. In the following quoted characterizations, features which apply to *A. gossypii* are italicized:

"Ascoideaceae—*Spore sacs (asci?) many spored;* gametangia, when present, sometimes multinucleate." Such genera as *Ascoidea* and *Dipodascus* are included here.

"Endomycetaceae—Asci with 8 ascospores or fewer; gametangia when present always uninucleate; *asci borne on a well developed mycelium.*" This includes the genera *Endomyces* and *Eremascus.*

"Saccharomycetaceae—Mycelium lacking, reproduction by budding, *asci formed by transformation of a single cell,* or as the result of fusion of two cells." Such genera as *Saccharomyces, Pichia,* and *Nematospora* are included in this family.

Recently, Routien (1949) has described a new genus, *Crebothecium,* designed to include the organism now known as *Eremothecium ashbyii. Ashbya gossypii* closely resembles *E. ashbyii* and with the exception of the morphology and arrangement of the spores within the sporiferous sacs, the two species could well be included in the same genus. The possibility that *A. gossypii* actually represents a species within the genus *Eremothecium,* or the new genus *Crebothecium,* if such genus is valid, is not without consideration. There is the further possibility that *E. ashbyii* could represent a species within the genus *Ashbya* since the latter has been validly described. Guilliermond (1936) has pointed out the essential differences between *E. ashbyii* and *A. gossypii.* The former organism has broader, shorter spores which are curved at the needle-like extremity, beveled at the opposite end, and lack elongate appendages. They are not arranged in any predetermined order, *i.e.,* there are no fascicles of spores. In addition, the central portion of the spores of *E. ashbyii* give a positive test for pectin, while those of *A. gossypii* do not. Septa are more numerous in *A. gossypii* than in *E. ashbyii,* whereas the dichotomy of the mycelium of the latter is more regular. *E. ashbyii* appears to be more fastidious in its nutritional requirements. According to the philosophy of the investigator, these differences could be regarded or disregarded in establishing nomenclature.

Further study of the characteristics and relationships of these and related genera seems warranted not only from an academic but also from a practical standpoint, since certain of the species within this group are of considerable economic importance, patents covering the production of riboflavin being centered around particular species.

CULTURAL AND MORPHOLOGICAL CHARACTERISTICS

The cultural characteristics of *Ashbya gossypii* have been described by a number of investigators, including Nowell (**1917**), Ashby and Nowell (**1926**), Guilliermond (**1927, 1936**), Stelling-Dekker (**1931**), Wallace (**1932**), Frazer (**1944**), and Wickerham *et al.* (**1946**). The following characterization is based upon these reports. *A. gossypii* grows freely on potato, potato-glucose agar, potato-sucrose agar, beer-wort, gelatin, and carrot, producing at first a spreading, moist, appressed hyphal growth with short, pointed, matted hair-like outgrowths over the surface. After a week on potato, scattered pustular dots appear which unite to form a folded, vermiform, firmly gelatinous plechtenchyma. The pigmentation of the substrate remains unchanged. On potato-glucose or potato-sucrose agar a similar hyphal growth appears which changes to a moist, translucent, dirty-white, folded and wrinkled plechtenchyma which can be readily peeled off.

Based upon our studies of a flavinogenic strain (fig. 1A) of the organism, the following colony description may be taken as typical of riboflavin producing strains:

Colonies upon yeast extract-malt extract-peptone-glucose agar after 10 days at room temperature (24–26° C), circular, flat to slightly raised, membranous, myceloid with advancing edge filamentous. On the second or third day of growth short, pointed, matted, hair-like growths form on the surface of the colony. On the fourth or fifth day, radial ridges or grooves appear, and in some instances haphazard wrinkles develop. Colonies grow at a fairly uniform rate of 3 to 5 mm. per day, reaching a maximum diameter of 38 to 50 mm. in 10 days, usually 35 to 40 mm. The marginal edges of colonies are made up of typical dichotomously branched hyphae (fig. 2B). Colonies, at first, completely white to greyish-white, becoming pale lemon yellow in 2 to 3 days, most intense at the center. At 5 days, the pigment is intensified to a lemon yellow and at 10 days to an orange yellow to reddish orange. The advancing edges of the colonies at all times are white for approximately 5 mm. Depending upon the rapidity of riboflavin synthesis on solid media, concentric rings begin to appear marking zones of periodically increased flavinogenesis. Sectoring to white areas is infrequently noted. In 7 to 10 days, the medium surrounding the colony is often pigmented a yellowish-green due to the diffusion of riboflavin or some derivative. Colonies are extremely adherent and may be peeled from the surface of the medium in their entirety.

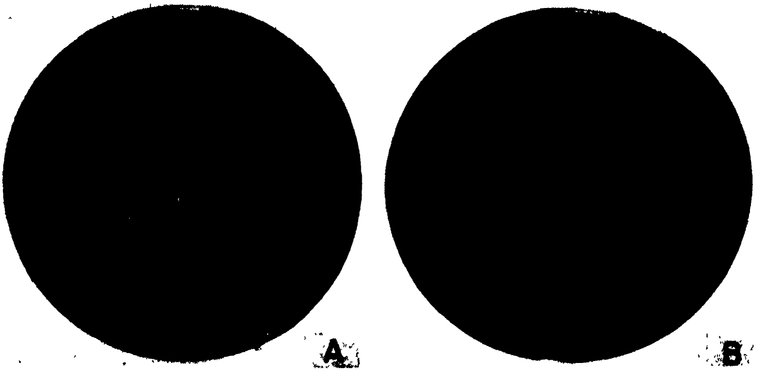

Fig. 1. *Ashbya gossypii* (Ashby and Nowell) Guilliermond. Ten-day colonies growing on peptone, yeast extract, malt extract, glucose medium; room temperature. *A*, Strain NRRL Y-1056 (flavinogenic). *B*, Strain NRRL Y-985 (non-flavinogenic).

In our experience non-flavinogenic strains of *Ashbya gossypii* duplicate the above except for the absence of pigmentation and a reduced tendency toward the formation of radial ridges, grooves, or wrinkling (FIG. 1B).

In wort and other liquid media, mycelial flocs are generally formed in a clear liquid. A slimy precipitate has been observed in some cases. Under agitation and aeration in liquid media, the culture becomes very homogeneous and tends to rather rapid autolysis after 7 days. Flavinogenic strains are characterized by the production of lemon yellow to orange pigment in certain media most intense when cultures are under aeration.

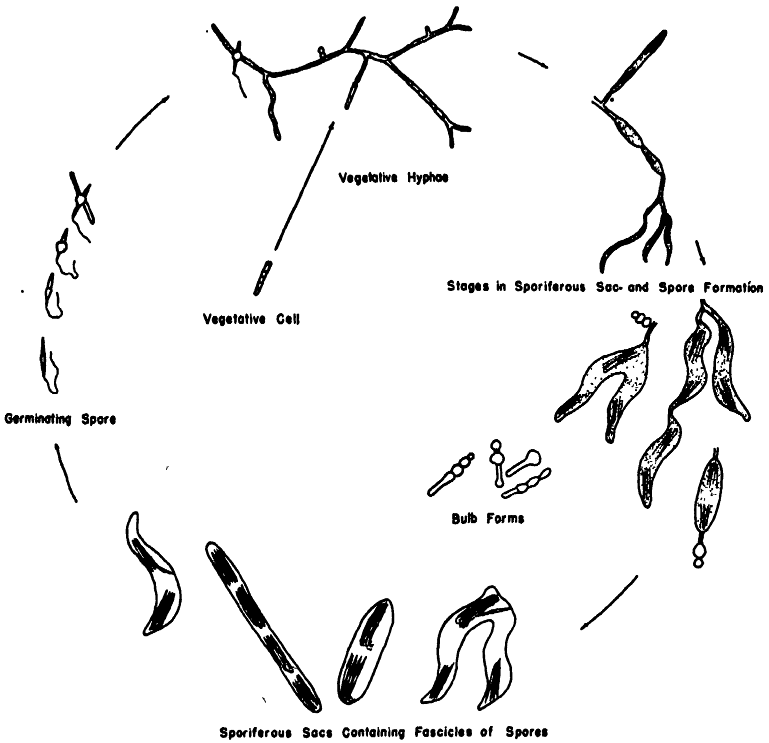
Vegetative Hyphae
Stages in Sporiferous Sac- and Spore Formation
Vegetative Cell
Germinating Spore
Bulb Forms
Sporiferous Sacs Containing Fascicles of Spores

FIG. 2. Schematic presentation of the life-cycle of *Ashbya gossypii* (Ashby and Nowell) Guilliermond.

Descriptions of the morphology of *Ashbya gossypii* have been recorded by Nowell (1917), Ashby and Nowell (1926), Guilliermond (1927, 1928, 1936), Stelling-Dekker (1931), and Wallace (1932). From these studies and our own observations, the following morphological characteristics may be considered as typical of this organism (FIGS. 2, 3, 4):

Hyphae hyaline, often vacuolated or containing a granular material and numerous hyaline droplets, at first non-septate. The first septum develops 150 to 200 μ behind the growing tip, others forming at intervals of 50 to 70 μ. Hyphae converging slightly at the septa, developing into long thin segments from 6 to 10 μ in diameter, branching dichotomously. Lateral buds developing on the hyphae at an early stage of growth, singly or in short chains, falling away and reported to be capable of germination. Vegetative reproduction reported to occur through transverse fission and subsequent separation of the daughter cells, this toruloid form being assumed only under conditions which preclude spore formation. In flavinogenic strains, riboflavin or a derivative may be observed within certain cells as a yellowish-oily fluid, or as needle-like orange crystals (FIG. 3D). The number of nuclei in the cells varies.

Sporiferous sacs (asci?, sporangia?) abundant, developing from hyphal segments, appearing haphazardly along the mycelium, occurring singly, or in chains, sometimes branched. These cells vary considerably in size, but range predominantly from 100 to 200 by 10 to 20 μ. They occur in a variety of shapes, sometimes clavate or cylindrical, but most frequently in the form of a sigmoid body. Glycogen is abundant at the beginning of spore formation and the sporiferous sacs may contain a granular protoplasm or may be highly vacuolated. Later, the interior of the sacs becomes hyaline except for the space occupied by the spores. The sacs ultimately break or autolyze to release fascicles of mature spores.

The nuclei in the sacs are reported to undergo two mitoses in spore formation similar to those of the higher Ascomycetes. Guilliermond (1927, 1928) has described in detail the cytological processes involved. At the end of the nuclear divisions, a sporogenous plasma remains. This material then divides into long filaments, reuniting into fascicles as the spores are formed.

Mature spores are grouped parallel into two or more fascicles of 2 to 6 spores, and are arranged lengthwise in the sac. The spores, when liberated, are held together by means of long intertwined appendages 50 to 100 μ in length, consisting of a difficultly stainable homogeneous plasma. Spores may number from 4 to 32 per sac (usually 12 to 16). Dependent upon the substrate in or upon which the organism has been cultivated, spores vary in dimensions from 25 to 37 by 2 to 5 μ. The spores are acicular to fusiform. They are described by Guilliermond as having an anterior region occupied by structures resulting from a degeneration of the cytoplasm, a central region made up of very dense cytoplasm containing the nucleus and giving a negative test for pectin, and a polar region consisting of the filiform appendage. In the center of the spore a thin septum may be observed occasionally. In germinating, a globular expansion occurs in the central nucleate region and from this arise one or more germ-tubes which ultimately develop into hyphae (FIGS. 2, 3).

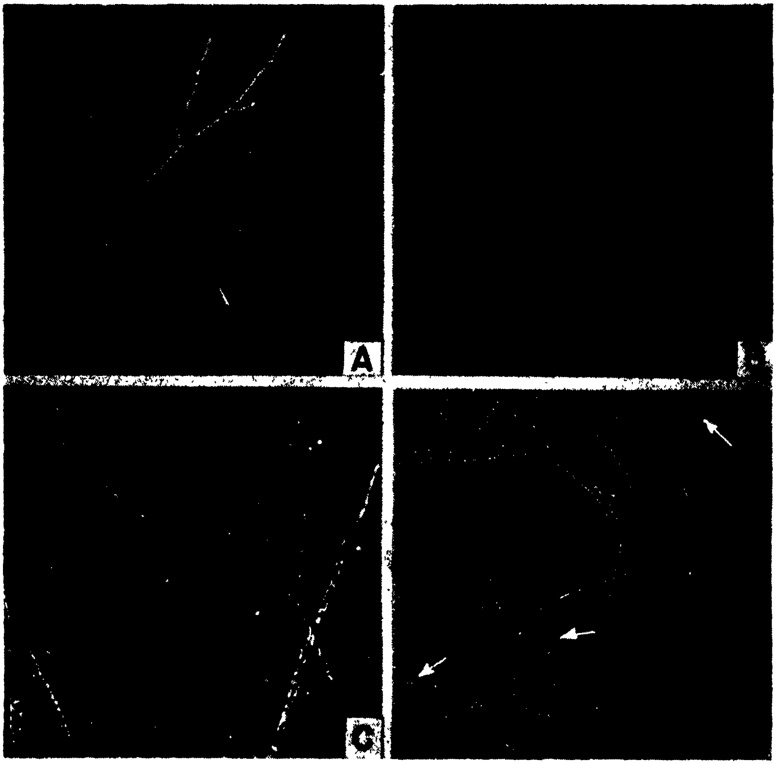

FIG. 3. *Ashbya gossypii* (Ashby and Nowell) Guilliermond (Strain NRRL Y-1056). *A*, *B*, and *C*, Preparations from 10-day colony, room temperature. *A*, Germinating spore, × 750. *B*, Typical dichotomous branching of hyphae, edge of colony, × 200. *C*, Typical hyphae showing septa, dichotomous branching, and granular nature of contents, × 1500. *D*, Typical preparation from fermented substrata showing crystals of riboflavin outside cells (arrows) and "bulb-forms" (upper center), × 750.

Swollen hyphal elements, termed "Bulb-forms" (FIGS. 2, 3, 4), are typical of the flavinogenic strains of *Ashbya gossypii*. These occur in cultures where riboflavin has been synthesized. They possibly represent the response of the organism to changes in osmotic tension influenced by the production of large amounts of the vitamin. These cells are usually filled with a yellowish-oily fluid, probably riboflavin or some derivative in the dissolved state.

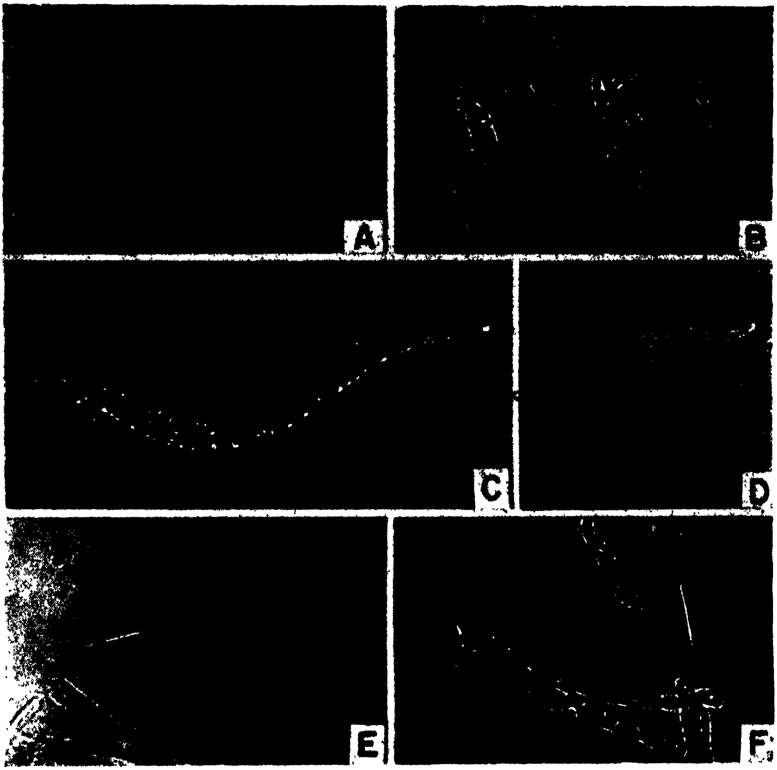

FIG. 4. *Ashbya gossypii* (Ashby and Nowell) Guilliermond (Strain NRRL Y-1056). Preparations from 10-day colony, room temperature. *A* and *B*, typical mounts showing nature of hyphae, immature sporiferous sacs, "bulb-forms" and sporiferous sacs containing spores, × 300. *C*, Sporiferous sacs at end of chain, showing characteristic sigmoid form and granular nature just prior to spore formation, × 1500. *D*, Sporiferous sac containing one fascicle of spores, second fascicle has been released through pore at lower end of sac, × 1300. *E*, Single spore, showing septum and portion of appendage, × 1300. *F*, "Bulb-forms," × 1300.

They vary considerably in size and appearance and develop either as terminal or intercalary cells. In some cases there is a distinct resemblance to chains of yeast-like cells.

PHYSIOLOGICAL CHARACTERISTICS

The nitrogen requirements of *Ashbya gossypii* have been investigated rather extensively. Farries and Bell (1930) found that KNO_3 and NH_4^+ salts were of little value as nitrogen sources. Their findings were confirmed by Buston *et al.* (1938) who observed no utilization of the ammonium salts of sulfuric, nitric, hydrochloric, tartaric, lactic, or pyruvic acids in mineral-salts media containing inositol and a factor obtained from lentils by Buston and Kasinathan (1933). When these media were supplemented with β-alanine or *l*-aspartic acid, moderate growth was attained. Stelling-Dekker (1931) and Wickerham (personal communication) have observed that NO_3^- is not assimilated by either flavinogenic or non-flavinogenic strains.

Farries and Bell (1930) further demonstrated that the organism is capable of growing in media containing the hydrolytic products of natural proteinaceous materials such as degraded animal protein, egg-white, gluten, or casein. Their strains failed to grow in media containing gelatin, edestin, or fibrin in any form, or in media containing simple mixtures of amino acids. Growth was obtained with certain of these latter materials when peptone, or a factor easily separable by alcoholic precipitation from egg-white, or casein was present. Buston and Pramanik (1931) and Buston *et al.* (1938) in confirming these findings identified one of the accessory factors as *meso*-inositol. With media containing *meso*-inositol and the lentil factor of Buston and Kasinathan (1933), *Ashbya gossypii* was shown capable of utilizing simple mixtures of amino acids, asparagine, or ammonium aspartate as nitrogen sources. Tryptophane was found to be non-essential. Stelling-Dekker (1931) has reported that the organism can feebly attack gelatin, as shown by liquefaction after 42 days' incubation.

It was ultimately determined in a series of studies by Buston and Pramanik (1931), Buston and Kasinathan (1933), Kögl and Fries (1937), Fries (1938), and Schopfer (1944) that in addition to *meso*-inositol, biotin and thiamin were required for adequate growth of *A. gossypii.* Of these factors, biotin (the lentil factor of Buston and Kasinathan) was found to be the most important. Thiamin is synthesized by the organism, but in sub-optimal amounts. Good growth was obtained in glucose-mineral salts media in the presence of the three factors. Attempts to replace the biotin with pimelic acid or with a mixture of pimelic acid and *l*-cystine have been unsuccessful (Robbins and Ma, **1942**).

The biotin requirement is such that a microbiological assay for this factor has been developed, based on the fact that within certain ranges, the weights of mycelial pads are directly proportional to the concentration of biotin in the medium (Robbins and Schmidt, **1939**; Robbins, **1940**). Their basal medium consists of dextrose, asparagine, mineral-salts, *meso*-inositol, thiamin, and trace elements. For biotin assay, cultures were incubated at 23 to 25° C for 9 days, the mats weighed, and the biotin content of samples calculated from a standard curve.

Ashbya gossypii has also proved useful in other physiological studies, *e.g.,* the mechanism of the physiological activity of gammexane (hexachlorocyclohexane). The γ-compound was shown to markedly decrease growth of the organism while the α- and β-isomers had little effect. This confirmed the hypothesis that the activity of the γ-isomer is due to its antagonism toward the essential metabolite *meso*-inositol, considered to have the same spatial configuration (Buston *et al.,* **1946**). Hawker (**1948**) has employed *A. gossypii* in mixed culture with *Melanospora destruens* to prove the latter capable of synthesizing inositol. Buston and Roy (**1949**) have used the organism in a study of the mechanism of action of certain unsaturated lactones. Their evidence indicates that the inhibition of growth caused by these compounds is not concerned with the metabolism of thiamin, ascorbic acid, nicotinic acid, biotin, or inositol. They further demonstrated that certain sulfhydryl compounds and amino acids may counteract the effects of these lactones.

The carbon requirements of *Ashbya gossypii* have been studied by several investigators. Marsh (**1926**) observed that the organism was unable to attack potato starch in a mineral-salts medium.

Ashby and Nowell (**1926**) reported it as having little diastatic ability and Farries and Bell (**1930**) and Wickerham (personal communication) have indicated that the organism may attack soluble starch only weakly. Glucose, fructose, and sucrose have been shown to serve as adequate carbon sources. Maltose and cellobiose are assimilated less readily. Rhamnose, arabinose, xylose, galactose, lactose mannitol, and inulin, however, do not serve as adequate carbon sources in peptone or amino acid-mineral salts-vitamin media (Farries and Bell, **1930**; Wickerham, personal communication).

Stelling-Dekker (**1931**) has reported that *A. gossypii* is unable to ferment glucose, fructose, mannose, galactose, sucrose, maltose, or lactose, when measured in terms of gas production. Ethyl alcohol was shown to support slight growth when employed as a substrate.

Marsh (**1926**) and Pearson (**1947**) have reported that the organism is unable to attack cellulose.

Farries and Bell (**1930**) observed traces of ethyl alcohol in certain media in which *A. gossypii* has been cultivated.

Mickelson (**1948**) has stated that the end products of glucose metabolism of *A. gossypii* are cells and CO_2. He further indicated that pyruvate, acetate and ethyl alcohol are readily oxidized, whereas lactate and succinate are only slowly oxidized.

RIBOFLAVIN PRODUCTION

As a physiological function of potentially great economic importance, certain strains of *Ashbya gossypii* possess the ability to synthesize large quantities of the vitamin riboflavin. As early as 1930 Farries and Bell noted the production of yellow pigment by certain strains, whereas it was not identified as riboflavin until 1935 (Guilliermond *et al.*). Until 1946, only traces of pigment were observed, principally in old cultures. It remained for Wickerham *et al.* (**1946**) to demonstrate substantial riboflavin synthesis in a strain earlier received from Dr. W. J. Robbins, New York Botanical Gardens. Following this observation and report, *Ashbya gossypii* NRRL Y-1056 has been developed by Tanner and coworkers (**1947**, **1948**, **1949**) and by the authors (**1949**) to a point where yields up to 1760 μg. of riboflavin per ml. have been obtained in shake-flask cultures in a peptone-corn steep liquor-glucose medium. These maximum yields have been attained through feeding additional glucose during the course of the fermentation (Pridham, **1949**). Yields of 500 to 600 micrograms per ml. are regularly achieved in pilot-plant trials by submerged aerobic fermentation using animal stick liquor, corn steep liquor, and commercial glucose (Tanner *et al.*, **1947**, **1948**, **1949**; Langford, **1948**; Pfeifer *et al.*, **1949**). Fermented media are ultimately dried down to give riboflavin concentrates containing from 25,000 to 30,000 μg. of the vitamin per gram. This material is employed directly in supplementing animal feeds. By appropriate chemical or microbiological techniques the riboflavin may also be recovered to meet pharmaceutical standards.

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PATHOGENIC SPOROTRICHA; THEIR CARBOHYDRATE REACTIONS

H. I. Lurie *

In 1809, Link (**10**) described eleven species of *Sporotrichum,* most of which were derived from decaying wood.

It was not until 1898 that Schenck (**16**) described a fungus resembling a *Sporotrichum* isolated from a human lesion. One year later Brayton (**4**) reported a case which clinically accorded with sporotrichosis but cultural examination was not done. In 1900, Hektoen and Perkins (**9**) isolated from a patient a fungus which was found to be identical with that of Schenck. They named the organism *Sporotrichum Schenckii.*

Three years later de Beurmann and Ramond (**5**) described the first case of sporotrichosis in France. This strain was studied by Matruchot and Ramond (**11**). They named it *Sporotrichum Beurmanni.* During the next few years numerous cases were reported in the world literature.

Several pathogenic species of *Sporotrichum* have been reported. Some descriptions include the carbohydrate fermentations, *e.g., S. Grigsbyi* is said to be inert in glucose, sucrose and lactose, while *S. Fonsecai* is said to produce acid with glucose, fructose and maltose. Schenck (**16**) as well as Hektoen and Perkins (**9**) report that *S. Schenckii* produces no gas in lactose, glucose or saccharose broth. Acid production is not mentioned. Gougerot and Blanchetière (**8**) state that all *S. Beurmanni* strains studied by them ferment saccharose but not lactose and that the original *S. Schenckii* strain hydrolyses and ferments lactose but not saccharose.

In 1915 Meyer and Aird (**12**) carried out an extensive investigation of the carbohydrate fermentations of fifteen strains of *Sporotrichum.* When grown in Erlenmeyer flasks for 60–90 days in the presence of a litmus indicator all strains were found to produce acid but no gas in glucose. No strains fermented lactose or mannite. Other sugars gave variable results. When twelve of the strains were grown for several months, the acid produced was estimated by titrating with N/20 NaOH. All were found to ferment glucose with the production of lactic acid. Lactose was not fermented by any strain in 68 days, but after 270 days all strains produced a trace of acid. *S. Beurmanni* did not ferment saccharose. They conclude by saying that the differentiation of species by carbohydrate fermentations is impossible.

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In many reports the technique adopted to determine whether a sugar was fermented or not is not described. Most workers depend on the production of gas and the change of pH to acid as shown by indicators. In a few of the more intensive studies the total amount of free acid was estimated by titration with dilute alkali and in one or two cases the acid was identified.

Since ammonia and potassium bicarbonate are catabolic products of fungus growth both the pH of the medium as shown by indicators and the total amount of free acid as shown by titration will depend on the balance between the amount of acid produced in the fermentation of a carbohydrate and the amount of catabolic products excreted by the growing fungus. It is possibly due to these factors that conflicting reports appear in the literature.

With this in mind, it was thought that a more detailed approach was necessary.

Seven strains of *Sporotrichum* were selected for this study:

No. 8. *S. asteroides.* Splendore. Westerdijk, Baarn. June 1941.
No. 13. *S. Schenckii.* Duke University. February 1944.
No. 20. *Rhinocladium Beurmanni.* Dr. Langeron, Paris. 1934.
No. 21. *Rhinocladium equinum.* Sabouraud's collection. 1934.
No. 32. *S. Beurmanni.* South Africa. Isolated from patient who contracted disease in gold mine. 1947.
No. 61. *S. Beurmanni.* South Africa. Isolated from timber in same gold mine. 1947.
No. 72. *S. tropicale.* Dr. Ghose, India.

The first experiment was to note changes in pH of the medium as shown by color changes of indicators. Various media and indicators were tried. A medium containing Casamino-acids with

TABLE 1

ACID PRODUCTION OF SPOROTRICHUM SP. WITH VARIOUS CARBOHYDRATES ON A SOLID HYDROLYSED CASEIN MEDIUM. ORIGINAL pH = 6.8. BROMO-CRESOL-PURPLE INDICATOR. 37°C

Strain	4 days							5 days						
	Glycerine	Glucose	Mannite	Saccharose	Maltose	Lactose	Starch	Glycerine	Glucose	Mannite	Saccharose	Maltose	Lactose	Starch
8	++	±	+	–	–	–	–	++	±	+	–	–	–	–
13	±	+	+	–	–	–	–	+	++	+	–	–	–	–
20	+	±	+	–	–	–	–	++	±	++	–	–	–	–
21	++	++	++	–	–	–	–	++	++	++	–	–	–	–
32	++	++	++	–	–	–	–	++	++	++	–	–	–	–
61	+	+	–	–	–	–	–	++	++	–	–	–	–	–
72														
	7 days							9 days						
8	++	–	±	–	–	–	–	++	–	–	–	–	–	–
13	+	+	±	–	–	–	–	++	+	–	–	–	–	–
20	++	–	+	–	–	–	–	++	–	±	–	–	–	–
21	++	++	++	–	–	–	–	++	++	++	–	–	–	–
32	++	++	++	–	–	–	–	++	++	++	–	–	–	–
61	++	+	–	–	–	–	–	++	+	–	–	–	–	–
72														
	10 days							15 days						
8	++	–	–	–	–	–	–	++	–	–	–	–	–	–
13	++	–	–	–	–	–	–	++	–	–	–	–	–	–
20	++	–	–	–	–	–	–	++	–	–	–	–	–	–
21	++	++	++	–	–	–	–	++	–	–	–	–	–	–
32	++	++	++	–	–	–	–	++	++	++	–	–	–	–
61	++	–	–	–	–	–	–	++	–	–	–	–	–	–
72														
	21 days							24 days						
8	++	–	–	–	–	–	–	++	–	–	–	–	–	–
13	++	–	–	++	++	–	–	++	–	–	++	++	–	–
20	++	–	–	–	–	–	–	++	–	–	–	–	–	–
21	++	–	–	–	–	–	–	++	–	–	–	+	–	–
32	++	++	++	–	–	–	–	++	++	++	–	–	–	–
61	++	–	–	++	–	–	–	++	–	–	++	–	–	–
72	–	–	±	–	–	–	–	–	–	+	–	–	–	–
	28 days							31 days						
8	++	–	–	–	–	–	–	++	–	–	–	–	–	–
13	++	–	–	++	++	–	–	++	–	–	+	±	–	–
20	++	–	–	–	–	–	–	++	–	–	–	–	–	–
21	++	–	–	–	++	–	–	++	–	–	–	++	–	–
32	++	++	++	–	–	–	–	++	++	++	–	–	–	–
61	++	–	–	–	–	–	–	++	–	–	–	–	–	–
72	–	–	±	–	–	–	–	–	–	+	–	–	–	–

++ = strongly acid. + = acid. ± = trace of acid. – = no acid.

l-cystine, nicotinic acid and agar was found to give the most satisfactory growth. Bromo-cresol-purple was used as an indicator. At a pH of 6.8 it is a definite purple color whereas below pH 6 it is bright yellow. Into the basic medium were incorporated various carbohydrates in a concentration of 1 per cent. The pH in each case was adjusted to 6.8. Slopes were inoculated with the different strains of *Sporotrichum* and maintained at 37° C. All cultures were examined daily and the color changes recorded. The results are summarized in table 1.

Strain No. 72 showed very poor growth. It will be seen that of the other six strains, all produced acid in glycerine and glucose, five in mannite, two each in saccharose and maltose and no strain produced acid in lactose or starch.

It is worthy of note that whereas on the fifth day all strains (except No. 61 in mannite) showed the presence of acid in glycerine, glucose and mannite, by the fifteenth day glycerine was still acid with all strains, whereas glucose and mannite had reverted to their original pH with all strains except No. 32. It will also be seen that acid appeared for the first time in maltose and saccharose (strains 13 and 61) only after twenty-one days.

As the changes in pH appeared to vary with the length of time, it was thought advisable to see if these results were reproducible. Consequently the experiment was repeated and a few more carbohydrates were included. For the second experiment strain No. 72 was kept at room temperature and a satisfactory growth was obtained. The results are summarized in table 2. All seven strains produced acid in glycerine, glucose, mannite and levulose, three strains produced acid in sorbite, dextrine, adonite and inulin, two produced a trace of acid in arabinose and dulcite.

By comparing tables 1 and 2 it will be seen that the results are not entirely comparable and that there is nothing constant about the time taken for the acid to appear or disappear, *e.g.*, in the first experiment strain No. 61 did not produce acid in mannite but it did do so in the second experiment. In the first experiment strain No. 20 produced acid in glycerine on the fourth day but in the repeat experiment it did not appear until the twelfth day. In the first experiment strain No. 21 showed much acidity in glucose from the fourth to the tenth day and by the fifteenth day the acidity had disappeared. In the second experiment acid was demonstrable on the fourth day only and had disappeared by the fifth day.

TABLE 2

ACID PRODUCTION OF SPOROTRICHUM SPP. WITH VARIOUS CARBOHYDRATES ON A SOLID HYDROLYSED CASEIN MEDIUM. ORIGINAL pH = 6.8.

BROMO-CRESOL-PURPLE INDICATOR. STRAINS 8 TO 61 AT 37°C. STRAIN 72 AT ROOM TEMPERATURE

Strain	3 days										5 days									
	Glycerine	Glucose	Mannite	Laevulose	Sorbite	Adonite	Dextrine	Inulin	Arabinose	Dulcite	Glycerine	Glucose	Mannite	Laevulose	Sorbite	Adonite	Dextrine	Inulin	Arabinose	Dulcite
8	++	−	−	+	−	−	−	−	−	−	++	−	−	−	−	−	−	−	−	−
13	++	++	−	++	−	−	−	−	−	−	++	++	−	++	−	−	−	−	−	−
20	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	±	−
21	++	+	−	++	−	−	−	−	−	−	++	−	+	++	−	−	−	−	−	−
32	++	++	++	−	++	−	−	−	−	−	++	++	++	++	++	+	−	−	−	−
61	++	++	−	++	−	−	−	−	+	−	++	++	++	++	−	−	−	−	±	−
72	−	−	−	+	−	−	−	−	−	−	++	++	−	++	−	−	−	−	−	−
	8 days										12 days									
8	++	−	−	−	−	−	−	−	−	−	++	−	±	−	−	−	−	−	−	−
13	++	++	±	++	−	−	+	−	−	−	++	++	−	++	−	−	++	−	−	−
20	−	−	−	−	−	−	−	−	−	−	++	++	±	−	−	−	−	−	−	−
21	++	−	++	++	++	−	−	−	−	−	++	−	++	++	++	−	−	−	−	−
32	++	+	++	++	++	++	−	−	−	−	++	−	++	++	++	++	−	−	−	−
61	++	++	++	++	−	−	−	−	+	−	++	++	−	++	−	−	−	++	−	−
72	++	++	++	++	++	−	++	−	−	−	++	++	++	++	++	+	++	−	−	−
	17 days										21 days									
8	++	−	±	−	−	−	−	−	−	−	++	+	++	−	−	−	−	−	−	−
13	++	++	−	++	−	−	++	±	−	−	++	++	−	++	−	−	++	++	−	−
20	++	++	±	−	−	−	−	−	−	−	++	++	±	−	−	−	−	−	−	−
21	++	−	++	++	++	−	++	−	−	−	++	−	++	++	++	−	++	−	−	−
32	++	−	++	++	++	++	−	−	−	−	++	−	++	++	++	++	−	−	−	−
61	++	++	−	++	−	−	−	++	−	−	++	++	−	++	−	−	−	++	−	−
72	++	++	++	++	++	++	++	−	−	−	++	++	++	+	++	++	++	−	−	−
	25 days										31 days									
8	++	++	++	−	−	−	−	−	−	−	++	++	++	−	−	−	−	−	−	−
13	++	++	−	++	−	−	++	++	−	−	++	++	−	++	−	+	++	++	−	±
20	++	++	+	−	−	−	−	±	−	−	++	++	++	−	−	−	−	−	−	±
21	++	−	++	++	++	−	++	−	−	−	++	−	++	++	++	−	++	−	−	−
32	++	−	++	++	++	++	−	−	−	−	++	−	++	++	++	++	−	−	−	−
61	++	++	−	++	−	−	−	++	−	−	++	++	−	++	−	−	−	++	−	−
72	++	++	++	−	++	++	++	−	−	−	++	++	++	−	++	±	++	−	−	−

++ = strongly acid. + = acid. ± = trace of acid. − = no acid.

It is possible, therefore, that some of the discrepancies in the literature are due to differences in the time the fungi are allowed to grow before the pH value is noted. These times vary with different authors from a few days to nine months. Obviously, therefore, the production of acid as shown by the color change of an indicator cannot be accepted as evidence of a carbohydrate being fermented unless numerous other factors are carefully standardized.

The titration of the amount of acid produced is subject to the same fallacies. The only certain method of determining whether a carbohydrate has been fermented or not is the actual estimation of the amount of carbohydrate present in a liquid medium before and after growth of the fungus in order to determine whether the total quantity has been significantly reduced. Unfortunately, the chemical methods which are available for the determination of individual sugars, and particularly of polyhydric alcohols, although accurate when only the sugar or polyhydric alcohol in question is present, are not completely specific. Other compounds which are capable of reducing alkaline copper reagents or of being oxidized by potassium dichromate, are also estimated by these methods. Hence any reducing substances of this kind which may be formed during the growth of the fungus will be measured in addition to the particular carbohydrate being tested. Because of this source of error a lower utilization of the carbohydrate would be indicated than actually occurred.

If this limitation is borne in mind, however, the method of direct determination of carbohydrates before and after growth of the fungus should afford useful information as to their utilization.

The following experiments were based on these lines. The different strains of *Sporotricha* were grown in a basic liquid medium at a pH of 6.8 with and without the addition of various carbohydrates. At the end of four weeks the pH was again estimated, the weight of fungus growth was determined and the residual carbohydrate estimated by a titrimetric method. The weight of growth in the basic medium was subtracted from the weight in the carbohydrate medium, the difference being taken as an indication of the stimulatory effect of the carbohydrate. In the case of each carbohydrate a control uninoculated flask was left standing for the same length of time. Its pH and carbohydrate content were determined as accurately as possible. The difference in pH units between the pH of the control medium and those in which the fungi had grown was recorded. The difference between the carbohydrate content of the control and the culture media was taken as an indication of the amount of carbohydrate utilized or altered by the fungus.

DETAILS OF TECHNIQUE

Basic medium

K_2HPO_4	1 gm.	
KCl	0.5 gm.	
$MgSO_4 \cdot 7H_2O$	0.5 gm.	
$FeSO_4$	0.018 gm.	
Sodium caseinate (Difco)	10 gm.	(Tested and found to be free from reducing substances)
Aq. dist.	1000 cc.	

A litre of water was heated to between 80 and 90° C and the inorganic salts added. The sodium caseinate was added slowly, during which time the mixture was stirred with a glass rod. When completely dissolved it was allowed to cool and was filtered through gauze. The volume was made up to 1000 cc. and the pH adjusted to 6.8; 50 cc. quantities were pipetted into each of eight 100 cc. Erlenmeyer flasks which were then autoclaved.

The carbohydrate medium was prepared in the same way except that the whole litre of medium was autoclaved, after which the carbohydrate was added to give a final concentration of 0.4 per cent. After dispensing 50 cc. quantities into sterile flasks, the flasks were steamed for one hour on three successive days.

The seven strains of fungus were maintained on Sabouraud's conservation agar containing no sugar. A platinum loop was drawn gently over the surface of the growth and a minimal amount of inoculum transferred to the respective liquid media. In each case a control flask was left uninoculated.

Whatman No. 42 filter papers were dried *in vacuo* over phosphorus pentoxide. Each filter paper was weighed in a weighing bottle, placed in an envelope and its weight recorded.

At the end of four weeks the content of each flask was filtered through these weighed filter papers using a Buchner funnel and slight suction. Each filter paper was then placed in a Petri dish and again dried and weighed. The *difference in weight was recorded as the weight of growth.*

The pH of the filtrate was estimated with a Beckman pH meter. The amount of carbohydrate present in each filtrate was determined titrimetrically. By subtracting the amount of carbohydrate in the various culture filtrates from that in the control, the amount of carbohydrate utilized or altered by the fungi was derived.

For each strain the weight of dry fungus produced by the basic medium was subtracted from that given by the carbohydrate medium. This gave some indication of the increase in growth due to the presence of the carbohydrate.

TECHNIQUE OF CARBOHYDRATE DETERMINATIONS

Monosaccharides (Modification of Benedict's method). An accurately measured 25 cc. of Benedict's reagent in a beaker is brought to boil. From a burette a standard 0.4 per cent solution of the particular carbohydrate in distilled water is added slowly until the blue color of the Benedict's reagent has almost disappeared. The mixture is boiled for three minutes until the reaction is complete. The carbohydrate solution is then added a few drops at a time, allowing ½ minute boiling after each addition. When the blue color is no longer visible a few drops of a 1 per cent solution of methylene blue is added to the mixture and the titration continued until all trace of blue has disappeared and a white precipitate remains.

After the Benedict's reagent is thus standardized, the procedure is repeated, titrating with the filtered medium. Usually, after a rough preliminary titration it was found necessary to use 10 cc. of Benedict's reagent and to dilute the medium 1:4 in order to obtain greater accuracy.

Disaccharides. To 25 cc. of a standard 0.4 per cent solution of the particular carbohydrate are added 15 cc. distilled water and 5 cc. $2N \cdot H_2SO_4$. The mixture is heated in a boiling water bath for five to ten minutes. After cooling, 5 cc. of $2N \cdot NaOH$ are added to neutralize the solution. The total volume is then made up to 100 cc. This hydrolyzed solution is titrated against Benedict's reagent in the same way as for monosaccharides. After the Benedict's reagent has been thus standardized the same procedure is applied to the filtered medium.

Polysaccharides. To 25 cc. of a standard 0.4 per cent solution of the particular carbohydrate are added 15 cc. distilled water and 15 cc. $2N \cdot H_2SO_4$. The mixture is heated in a boiling water bath for two and a half hours. From time to time a few drops of distilled water are added to replace that lost by evaporation. After cooling, 15 cc. $2N \cdot NaOH$ are added to neutralize the solution. The total volume is then made up to 100 cc. Thereafter the technique is the same as that for disaccharides.

Polyhydric alcohols. (Modification of A.O.A.C. method.) The following reagents are required:

1. Strong solution of $K_2Cr_2O_7$. 74.55 gm. pure dry $K_2Cr_2O_7$ dissolved in distilled water. Add 150 cc. conc. H_2SO_4, cool and dilute to 1 litre with distilled water.
2. Dilute solution of $K_2Cr_2O_7$. Dilute 50 cc. of the strong $K_2Cr_2O_7$ to 1 litre with distilled water.
3. Diphenylamine indicator. Dissolve 1 gm. diphenylamine in 100 cc. conc. H_2SO_4.
4. Retarder. Dilute 150 cc. of syrupy phosphoric acid with 600 cc. of distilled water and add 250 cc. conc. H_2SO_4.
5. Ferrous ammonium sulphate solution. Dissolve 30 gm. crystalline ferrous ammonium sulphate in distilled water, add 50 cc. conc. H_2SO_4, cool, dilute to 1 litre with distilled water.

Reagents 1, 2, 4 and 5 are diluted 1 : 3 before use.

To 25 cc. of the filtered medium are added 15 cc. of a 15 per cent trichloracetic acid solution and 10 cc. distilled water. The mixture is stirred, allowed to stand for 10 minutes and filtered through filter paper. To 25 cc. of this filtrate are added 30 cc. strong $K_2Cr_2O_7$ solution followed by 24 cc. conc. H_2SO_4. The mixture is heated in a boiling water bath for 20 minutes, cooled and diluted to 250 cc. with distilled water.

The ferrous ammonium sulphate solution is standardized by taking 20 cc. and then adding 20 cc. of the retarder, 4 drops of the indicator and 100 cc. distilled water, and titrating with the dilute $K_2Cr_2O_7$ solution until the liquid assumes a dark green color. The dilute $K_2Cr_2O_7$ solution is then added drop by drop until the color changes from blue grey to deep violet. (Volume of $K_2Cr_2O_7$ used = x cc.)

Twenty cc. of the ferrous ammonium sulphate (+ 20 cc. retarder + 4 drops indicator + 100 cc. distilled water) are next titrated with the oxidized medium. (Volume of solution used = y cc.)

The amount of polyhydric alcohol present in the 25 cc. sample taken for analysis is: $.01/3 \times (30 - 250x/20y)$ gm. The medium was originally diluted to half its strength with distilled water and trichloracetic acid. Therefore the amount of polyhydric alcohol in 25 cc. of the medium is $.01 \times 2/3 \times (30 - 250x/20y)$ gm.

The results of this experiment are summarized in table 3.

An examination of this table shows the following points:

1. In the basic medium without any carbohydrate the growth was very poor and the pH of the medium became more alkaline.

2. In the presence of glycerine, glucose, maltose, levulose and saccharose with all seven strains the medium became more acid, the weight of growth was improved and the concentration of sugar in the medium lowered.

3. With lactose and starch the changes in pH varied with the different strains, the growth weight of all strains was increased and the concentration of the carbohydrate lowered. There does not appear to be any relationship between the change in pH, the stimulation of growth and the amount of carbohydrate utilized, *e.g.*, in the case of lactose with strains No. 21 and 61, the reaction became more alkaline, there was no significant increase in growth but the concentration of carbohydrate was definitely reduced. With strains No. 13 and 32 there was no change in pH, a moderate increase in growth and a lowering in the carbohydrate content. One strain, No. 20, acidified the medium; its growth stimulation was less than that of No. 13 and 32 but the carbohydrate was lowered to the same extent.

4. In the presence of arabinose the medium became more alkaline with all strains, the growth weight was slightly increased but the sugar concentration was definitely reduced.

5. In the case of inulin the pH with all strains except No. 72 became more acid, the growth weight was increased but the carbohydrate was insignificantly reduced (except with strains No. 21 and 32).

6. With dextrine the pH became more acid; the growth weight of strains No. 8, 20, 32 and 72 was not significantly increased, that of the other strains being only slightly increased; an insignificant reduction in the carbohydrate occurred with all strains.

7. With glycogen the pH response varied with the different strains, there was no appreciable increase in weight but the carbohydrate content was slightly lowered.

8. In the presence of mannite the pH response was variable, no increase in weight was detectable and there was an apparent increase in the carbohydrate content.

In order to determine whether any reducing substance was present in the catabolic products, the seven strains were grown in the same basic medium without any carbohydrate. At the end of four weeks the filtered media were subjected to the potassium dichromate technique. It was found that with several strains a reducing substance had appeared in the medium. As this technique is incapable of differentiating mannite from other reducing substances the figures given in table 3 represent the total amount of reducing substance present. It is not possible, therefore, to state whether the concentration of mannite had been reduced or not. It can, however, be seen that there was no improvement in the growth of the fungus. The probability is, therefore, that mannite was not fermented.

In the case of glycerine, the figures given must be regarded as approximate. The total quantity of glycerine utilized probably exceeds the estimated amounts.

If one can accept the production of acid, the increased growth and the reduction of the carbohydrate content as evidence that the carbohydrate has been fermented, then all seven strains fermented glycerine, glucose, maltose, levulose and saccharose. In the case of arabinose, although the pH with all strains became more alkaline, there does appear to have been a utilization of the sugar. With lactose, inulin, dextrine, starch and glycogen the results were variable and inconclusive. It must, however, be borne in mind that there are limitations to the accuracy of the techniques employed for the determination of these carbohydrates, especially since the amounts utilized were small relative to the amounts added

TABLE 3

CORRELATION OF CHANGE IN pH AND WEIGHT OF GROWTH WITH UTILIZATION OF VARIOUS CARBOHYDRATES. (50 CC. SYNTHETIC LIQUID MEDIUM + 0.4% CARBOHYDRATE. ORIGINAL pH 6.8. FOUR WEEKS GROWTH AT ROOM TEMPERATURE)

Strain	Basic Medium							
	pH series A	pH series B	pH series C	Average pH	Weight of growth in mgms. Series A	Weight of growth in mgms. Series B	Weight of growth in mgms. Series C	Average weight of growth in mgms.
Control								
No. 8	7.32	7.74	7.9	7.65	8	5.5	3.5	6
No. 13	7.32	7.8	7.9	7.67	8	3.5	3.5	5
No. 20	7.25	7.7	7.55	7.5	10	5.5	7.5	8
No. 21	7.18	7.9	8.15	7.74	9	3.5	7	6.5
No. 32	7.18	7.75	7.95	7.63	9	9	4	8
No. 61	7.2	7.85	7.95	7.67	12	7	6	8
No. 72	7.32	7.7	7.9	7.64	12	6.5	6.5	8

Strain	Glycerine					Glucose				
	pH after 4 weeks	Difference between pH of culture and pH of control	Weight of growth in mgms.	Difference between weights of growth in carbohydrate and basic media	Difference between carbohydrate content of culture and control in mgms.	pH after 4 weeks	Difference between pH of culture and pH of control	Weight of growth in mgms.	Difference between weights of growth in carbohydrate and basic media	Difference between carbohydrate content of culture and control in mgms.
Control	6.52					5.7				
No. 8	5.8	−.72	35	29	−26	5.53	−.17	28.5	22.5	−150
No. 13	5.95	−.57	38.5	33.5	−26	5.42	−.28	33	28	−110
No. 20	5.8	−.72	40.5	32.5	−36	5.5	−.2	32	24	−120
No. 21	6.12	−.4	31.5	25	−72	5.5	−.2	28	21.5	−130
No. 32	6.15	−.37	40	32	−26	5.5	−.2	28.5	20.5	−165
No. 61	6.15	−.37	41	33	−26	5.52	−.18	35	27	−180
No. 72	6.0	−.52	38.5	30.5	0	5.5	−.2	27	19	−150
	Mannite					Laevulose				
Control	7.23					6.26				
No. 8	7.3	+.07	11	5	+62	5.78	−.48	24	18	−130
No. 13	7.3	+.07	12	7	+28	5.6	−.66	34	29	−111
No. 20	7.2	−.03	13	5	+15	5.62	−.64	32	24	−160
No. 21	7.13	−.1	16.5	10	+42	5.63	−.63	28	21.5	−149
No. 32	6.99	−.24	13	5	+92	5.78	−.46	25	17	−111
No. 61	7.23	0	6.5	—	+18	5.75	−.51	27	19	−130
No. 72	7.13	−.1	18	10	+64	5.73	−.53	32	24	−149

TABLE 3—*Continued*

Strain	Dextrine					Inulin				
	pH after 4 weeks	Difference between pH of culture and pH of control	Weight of growth in mgms.	Difference between weights of growth in carbohydrate and basic media	Difference between carbohydrate content of culture and control in mgms.	pH after 4 weeks	Difference between pH of culture and pH of control	Weight of growth in mgms.	Difference between weights of growth in carbohydrate and basic media	Difference between carbohydrate content of culture and control in mgms.
Control	6.79					6.6				
No. 8	6.79	0	19	13	−10	6.11	−.49	35	29	− 6
No. 13	6.64	−.15	26.5	21.5	−15	6.15	−.45	37	32	− 2
No. 20	6.79	0	17.5	9.5	−10	6.15	−.45	42.5	24.5	− 2
No. 21	6.03	−.76	32	25.5	−12	6.12	−.48	31	24.5	−35.5
No. 32	6.29	−.5	21.5	13.5	− 9	6.35	−.25	33	25	−37.5
No. 61	6.32	−.47	31	23	− 4	6.4	−.2	44	36	−21
No. 72	6.21	−.58	22.5	14.5	− 5	7.4	+.8	23.5	15.5	−12
	Arabinose					Maltose				
Control	6.42					6.8				
No. 8	6.65	+.23	23	17	− 64	6.5	−.3	40	34	−40
No. 13	6.6	+.18	24	19	− 64	6.25	−.55	51	46	−56
No. 20	6.46	+.04	26	18	−145	6.75	−.05	48	40	−61
No. 21	6.54	+.12	21.5	15	− 72	6.05	−.75	40	33.5	−57
No. 32	6.48	+.06	27.5	19.5	−124	6.5	−.3	40	32	−78
No. 61	6.48	+.06	28	20	−124	6.5	−.3	44	36	−75
No. 72	6.6	+.18	26	18	−115	6.0	−.8	75	67	−79
	Lactose					Saccharose				
Control	7.0					7.3				
No. 8	7.15	+.15	21	15	−37	7.0	−.3	27	21	− 60
No. 13	7.0	0	33	28	−46	6.96	−.34	32.5	27.5	− 32
No. 20	6.85	−.15	25	17	−62	7.1	−.2	24	16	− 39
No. 21	7.3	+.3	15	8.5	−57	7.3	0	27.5	21	− 44
No. 32	7.0	0	37	29	−62	6.93	−.37	22.5	14.5	− 55
No. 61	7.15	+.15	19	11	−70	6.8	−.5	29	21	−125
No. 72	7.05	+.05	26	18	−59	7.28	−.02	30	22	− 46
	Starch					Glycogen				
Control	6.9					7.0				
No. 8	7.16	+.26	21.5	15.5	− 8	7.3	+.3	5.5	—	−14
No. 13	6.5	−.4	26	21	−20	6.63	−.27	20	15	−28
No. 20	7.12	+.22	23	15	−16	7.26	+.26	8	—	−33.5
No. 21	6.6	−.3	27.5	21	−19	6.73	−.27	13.5	7	−32
No. 32	6.6	−.3	26	18	−20	6.78	−.22	19.5	11.5	−17.5
No. 61	6.5	−.4	27	19	−36	6.78	−.22	16	8	−38
No. 72	6.0	−.9	25.5	17.5	−36	6.25	−.25	18	10	−34.5

to the medium. Lactose and starch are probably fermented to a slight extent, and possibly also dextrine, inulin and glycogen. It is almost certain that no strains ferment mannite. There is no clear-cut difference between the seven strains as regards their ability to ferment the carbohydrates.

While not all the carbohydrates were used in each of the three experiments, several interesting comparisons are possible.

1. Glycerine, glucose and levulose, according to experiment 3, appear to be fermented by all seven strains. In the two previous experiments on solid media much acid was produced with these three carbohydrates.
2. While no fermentation of mannite was demonstrable in experiment 3, acid was produced in both previous experiments.
3. In the first experiment only two strains showed the production of acid with saccharose and maltose. Yet in the third experiment all strains lowered the pH and appeared to ferment these sugars.
4. On solid media no strain produced acid with starch, yet in the liquid medium five strains produced an appreciable lowering of the pH.

DISCUSSION

In table 4 is summarized the available literature on the carbohydrate reactions of different species of *Sporotrichum*. It is clear that there are many conflicting results. However, most investigators, including this study, agree that pathogenic *Sporotricha* produce acid with glycerine, glucose, maltose and levulose. All except Blanchetière and Gougerot (2) agree that lactose is not fermented. They state that the original *Schenckii* strain definitely ferments lactose and that this is one of the important features in the differentiation of *S. Schenckii* from *S. Beurmanni.* Their statement has never been confirmed. Although the results of this study cannot definitely confirm or contradict this, no significant differences were shown by the *Schenckii* and *Beurmanni* strains. Blanchetiere and Gougerot (2) also maintain that *S. Beurmanni* ferments saccharose and that *S. Schenckii* does not. Most workers have confirmed this statement. In the present study there does

TABLE 4

SUMMARY OF AVAILABLE LITERATURE ON CARBOHYDRATE REACTIONS OF SPOROTRICHA

Author	Species	Glycerine	Glucose	Mannite	Lactose	Saccharose	Maltose	Laevulose	Galactose	Arabinose	Inulin	Dextrine	Starch	Dulcite	Adonite	Salicin	Xylose	Raffinose
Blanchetière and Gougerot (2)	Beurmanni	+	+	+	−	+	+		+		+	−		+				
Dodge (6)	Schenckii var. Beurmanni	+	+	−	−	?+	+				+	−	+	−				
Moore and Kile (13)	Beurmanni		+	−	−	+	+	+	+	−		+					+	
Walker and Ritchie (18)	? Beurmanni		+	+	−	+	+		+		+			−	−	−		+
Pijper and Pullinger (15)	Beurmanni		−	−	−	−	−	−	−	−	−	−		−	−			−
Panja, Dey and Ghose (14)	Beurmanni	+		−	−	+	+				+	−						
Ghose (7)	Beurmanni var. indicum		−	−	−	−	−	−		−	−			−		−		
Schenck (16)	Schenckii		−		−	−												
Blanchetière and Gougerot (2)	Schenckii				+	−												
Blanchetière and Gougerot, quoted by Wolbach, Sisson and Meier (19)	Schenckii	+	+		+						+							
Anderson and Spector (1)	? Schenckii		−	−	−	+	−											
Taylor (17)	? Schenckii		+	−	−	−	−											
Boggs and Fried (3)	? Schenckii		+	−	−	−	+											
Meyer and Aird (12)	Schenckii and Beurmanni	±	+	−	−	+ or −	+ or −	+	+									

TABLE 4—*Continued*

Author		Species	Glycerine	Glucose	Mannite	Lactose	Saccharose	Maltose	Laevulose	Galactose	Arabinose	Inulin	Dextrine	Starch	Dulcite	Adonite	Salicin	Xylose	Raffinose
Wolbach Sisson and Meier (19)		Councilmani	+	+	−	−	−					−			+				
Blanchetière and Gougerot, quoted by Wolbach, Sisson and Meier (19)		Gougeroti	+	+	−	−	+												
Dodge (6)		Grigsbyi		−		−	−												
Dodge (6) *M*		Fonsecai	+					+	+										
Panja, Dey and Ghose (14)		Tropicale	+	+	+ late	−	−	+	+	+	−	−	+			−		+	−
Blanchetière and Gougerot, quoted by Meyer and Aird (12)		All pathogenic	+	+	−	−	+	+	+	+				+					
This study	Experiment No. 1	Various species	+	+	+	−	+ or −	+ or −						−					
	Experiment No. 2	Various species	+	+	+				+		−	+ or −	+ or −		−	+ or −			
	Experiment No. 3	Various species	+	+	+ or −	+ or −	+	+	+		−	+	+	+ or −					

+ = acid. − = no acid.

not appear to be any difference between the two species. *S. Councilmani, Grigsbyi* and *tropicale* are said not to ferment saccharose, whereas *S. Gougeroti* does do so. In this study it was found that *S. tropicale* ferments this sugar.

With regard to mannite all except two investigators agree that this carbohydrate is not fermented. *S. tropicale* is said to be a late fermenter. None of the species investigated in this study, including *S. tropicale,* fermented mannite after twenty-eight days.

Carbohydrates other than those mentioned above have not yet been adequately investigated.

It is more than likely that the discrepancies are due to differences in technique and in the interpretation of what constitutes evidence of fermentation. The production of acid alone is obviously insufficient evidence. It is worthy of note at this stage that Dodge (6) says of *S. Fonsecai:* "No fermentation of sugars; acid with glucose, fructose and maltose."

CONCLUSION

It would appear that pathogenic *Sporotricha* ferment glycerine, glucose, maltose and levulose, but probably do not ferment mannite or lactose to an appreciable extent. With regard to the other carbohydrates information is as yet inadequate. It appears to be extremely unlikely that the carbohydrate reactions will prove to be of any value in the differentiation of species.

ACKNOWLEDGMENTS

My thanks are due to Dr. G. Buchanan, Deputy Director of the South African Institute for Medical Research, for his continued interest in this work, and to Dr. W. I. M. Holman for his invaluable advice on the chemical analyses.

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* Not read in the original.

NEW SPECIES OF CELLULOSE DECOMPOSING FUNGI. II

L. M. AMES *

(WITH 13 FIGURES)

The present contribution deals with fungi isolated either from military materiel and equipment or from other sources. However, the three species herein described are of importance, both from military and civilian viewpoints, because of their cellulose destroying characteristics. These species have been cultivated on maize meal and potato extract agar with a strip of cloth or filter paper added, with 2% agar-agar, as described in a previous paper [1] to insure uniformity of growth conditions for all species of the Chaetomiaceae that have been described recently or that will be described in my future work. Exceptions, when necessary, will be explicitly reported.

The writer thanks the following men for their kindness in sending the material which is the subject of this paper: Dr. G. W. Martin, University of Iowa; Dr. E. A. Bessey, Michigan State College; and Dr. J. C. Gilman, Iowa State College.

Chaetomium fibripilium sp. nov.

Viridoflavum vel gilvo-brunneum. Peritheciis mediae vel magnae magnitudinis, subglobosis vel ovatis, basi acuminatis, 260 × 230 μ (200–330 × 185–285 μ) ostiolatis, cum rhizoideis gracilibus paratis, interdum cum cirrhis. Pilis lateralibus numerosis, gilvo-viridantibus cum senectute gilvo-brunneis basi rectis, apicibus undulatis abnormis, septatis, basi 2.25–3.25 μ diametro, apicibus acuminatis. Pilis terminalibus numerosis, intertexis, gilvo-viridantibus, cum senectute gilvo-brunneis, simpliciter vel compositer ramosis, septatis, capitem frutectosum magnum formans, basi 2.25–3.5 μ diametro, apicibus undulatis abnormis et acuminatis. Ascis clavatis, octosporis, fugacibus. Ascosporis maturis dilute brunneis, ovatis, in ultima subapiculatis, 10 × 8 μ (9.5–11.25 × 7–8.5 μ).

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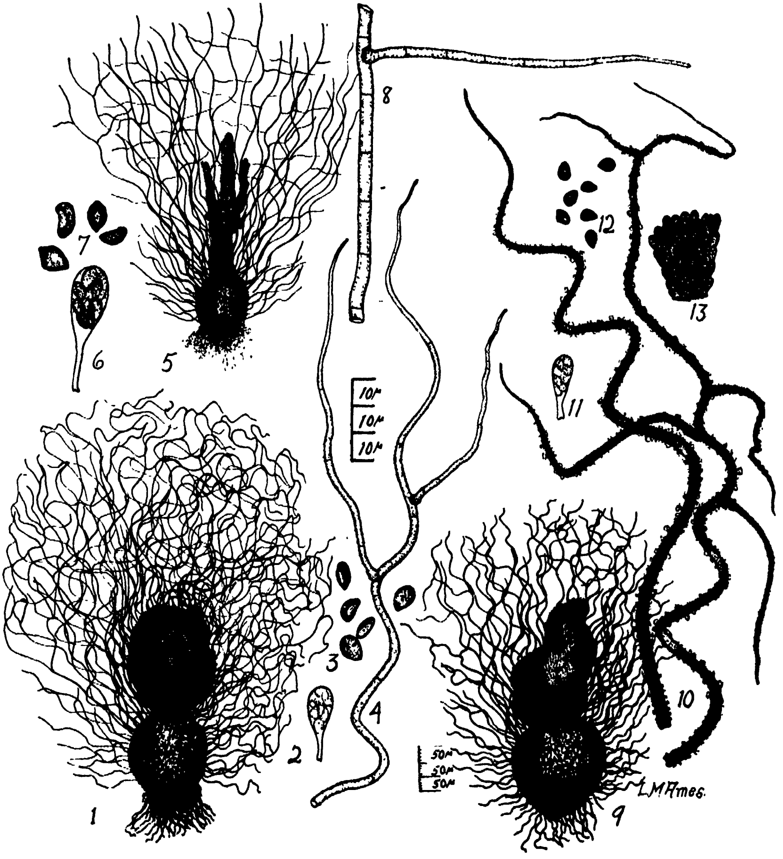
[1] Ames, L. M. New cellulose destroying fung[illegible]olated from military material and equipment. Mycologia 41: 637–648. [illegible]9.

Greenish yellow to yellowish brown. Perithecia medium to moderately large in size, subglobose or ovate with bluntly pointed bases 260 × 230 μ (200–330 × 185–285 μ), ostiolate, supported by delicate rhizoids, occasionally producing cirrhi. Lateral hairs numerous, greenish yellow, browning with age, straight near base, irregularly wavy toward the apex, distinctly septate, at base 2.25–3.25 μ



FIGS. 1–4, *C. fibripilium:* 1, mature perithecium· 2, immature ascus; 3, mature ascospores; 4, branched terminal hair. FIGS. 5–8, *C. mollipilium:* 5, mature perithecium; 6, mature ascus; 7, mature ascospores; 8, detail of branched terminal hair. FIGS. 9–13, *C. nigricolor:* 9, mature perithecium; 10, detail of two terminal hairs, one unbranched, the other branched; 11, immature ascus; 12, mature ascospores; 13, small group of young asci as seen when the perithecium is crushed, almost transparent in water mounts, this shown after staining.

in diameter, gradually tapering to the apex. Terminal hairs numerous, interwoven, greenish yellow when young, browning with age, unbranched or branched, some compositely branched, distinctly septate, forming a large bushy head, at base 2.25–3.5 μ in diameter gradually tapering to the apex. Asci club-shaped, 8-spored, fugaceous. Ascospores dilute green when young, when mature dilute brown, ovate, slightly apiculate at one end, round at the other, 10 × 8μ (9.5–11.25 × 7–8.5 μ).

Type. Isolated from sugar cane which was collected in the Hawaiian Islands. Culture was made by Dr. E. A. Bessey and sent to the writer for study.

C. fibripilium is distinguished, when young, by its neutral green ascospores which become pale brown with age, and its compact head of hairs, many of which are branched compositely. The branched terminal hairs are quite similar in structure to those of *C. sphaerale* Chivers, but differ in color, *C. sphaerale* being grayish-yellow to olive-yellow. The two species differ regarding the ostiole region, *C. sphaerale* having a distinct neck which is lacking in *C. fibripilium.*

Chaetomium mollipilium sp. nov.

Fulvum. Peritheciis magnitudinis mediae, globosis vel subglobosis, 175 × 130 μ (165–240 × 130–190 μ) ostiolatis, cum rhizoideis fulvis paratis, cum cirrhis. Pilis lateralibus, paucis, gracilibus, septatis, basi 3–3.75 μ, apicibus acuminatis. Pilis terminalibus moderate longis, gracilibus, septatis, basi 3.25–4.25 μ diametro, saepe ramosis, divaricatis, apicibus acuminatis. Ascis clavatis, octosporis, 38 × 12.5 μ, parte sporifera 27 μ. Ascosporis maturis fulvis, limoniformibus, utrinque apiculatis, 10–12.5 × 8.5–10 μ.

Light brown to cream-colored. Perithecia of medium size, globose to subglobose 175 × 130 μ (165–240 × 130–190 μ,), ostiolate, supported by light brown rhizoids, with cirrhi. Lateral hairs moderate in number, wavy, seldom branched, septate, graceful, at base 3–3.75 μ in diameter, gradually tapering to the apex. Terminal hairs sparse, forming a relatively open head, septate, often branched with wide angles, at base 3.25–4.25 μ in diameter, gradually tapering to a narrow tip. Asci clavate, eight-spored, 38 × 12.5 μ, spore part lemon-shaped, apiculate at both ends, 12 × 10 μ (10–12.25 × 8.5–10 μ).

Type. Isolated from a Japanese raincoat. Culture was made by Dr. G. W. Martin while he was at the Jeffersonville Quartermaster Depot, Jeffersonville, Indiana, and designated as J–587, APO, 565. A similar isolate was designated as J–905.

This species is distinguished by its soft, easily mashed hairs, many of which are branched with wide angles. Looking directly down on comparatively fresh cultures the outline of the perithecium is clearly visible due to the paucity of terminal hairs. This species has been isolated four times from diseased fingernails. Nail specimens from four sources were washed in 95 per cent alcohol, rinsed in sterile distilled water and then placed on mineral agar. The perithecia which developed were found growing on the nail specimens. This is reported only as an interesting observation of apparent human nail infection by a species of *Chaetomium.*

Chaetomium nigricolor sp. nov.

Nigrum. Peritheciis magnitudinis mediae, globosis vel subglobosis, 250 × 230 μ (200–285 × 190–260 μ) ostiolatis, cum rhizoideis brunneis paratis, interdum cum cirrhis. Pilis lateralibus paucis gracilibus, obscure septatis, apicibus acuminatis, diametro 3.5–4.5 μ, scabris. Pilis terminalibus longis, numerosis, moderate rigidibus, scabridis, obscure septatis, compositer vel simpliciter ramosis, basi 3–4 μ diametro, apicibus acuminatis. Ascis clavatis, octosporis, 25 × 9 μ, parte sporifera 14 μ. Ascosporis maturis brunneis, amigdalo-formibus, apice subapiculatis, 5–6 × 4–5 μ.

Black. Perithecia of moderate size, globose to subglobose, 250 × 230 μ (200–285 × 190–260 μ), ostiolate, attached to the substratum with brown rhizoids, seldom provided with cirrhi. The bushy haired heads have a ragged appearance. Lateral hairs moderately numerous, straight or undulating, obscurely septate, 3.5–4.5 μ in diameter, roughened, black in color and tapering to faded tips. Terminal hairs numerous, black, regularly and thickly covered with little black projections, with larger crystals or projections appearing at random, undulating or with occasional spiral coils, frequently branching, at base 3–4 μ in diameter, tapering to faded tips. Asci fugaceous, club-shaped, 8-spored, 25 × 9 μ, spore part 14 μ. Mature ascospores brown, almond-shaped, slightly apiculate at one end, 5–6 × 4–5 μ.

Type. From India. This species was received from Dr. J. C. Gilman, who received the specimen from India under the binomial *Chaetomium convolutum* Chivers. They are similar only in color. *C. nigricolor* is recognized by the black, intermingling branched and simple undulating terminal hairs, and the small, brown ascospores.

STUDIES IN THE GENUS CINTRACTIA. II. C. AXICOLA AND RELATED SPECIES

LEE LING

(WITH 1 FIGURE)

Cintractia axicola (Berk.) Cornu, as the type of the genus, is one of the most widely distributed and best known species. Very little confusion has occurred concerning it in recent literature. However, a related species occurring on *Cyperus, Cintractia limitata* Clint., has occasioned much confusion. In the present paper, descriptions and synonymy of five species of *Cintractia* infecting *Fimbristylis* and *Cyperus* are presented. Besides the names included here, two other species, *Cintractia tangensis* P. Henn. and *Cintractia javanica* Racib., have been recorded on *Cyperus*. The former has not been available for study and hence has been excluded. The host of the latter was found to be a *Rhynchospora*. As in the previous series, the institutions in which the specimens examined are located are indicated by abbreviations. Abbreviations, which were not given in the previous paper, are as follows: BR = Jardin Botanique de l'Etat, Brussels; CMI = Commonwealth Mycological Institute, Kew; LP = Instituto de Botanica "Spegazzini," Universidad Nacional de La Plata; ND = Indian Agricultural Research Institute, New Delhi. Wherever the location is not given, the specimen is in the writer's personal collection. The writer is very grateful to those who furnished the material and facilities for study in their institutions; and to Mr. J. A. Stevenson for the review of the manuscript.

CINTRACTIA AXICOLA (Berk.) Cornu, Ann. Sci. Nat. Bot. VI. **15**: 279. 1883

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Cintractia mundkuri Chowdhury, Indian Jour. Agr. Sci. **14**: 231. 1944.
Cintractia peribebuyensis Speg. var. *major* Pavgi & Mundk. Indian Phytopath. **1**: 109. 1948.

Sori at the base of primary or auxiliary rays, less often in the ovaries, globoid to oblong, 1–4 mm. in length, at first each covered by a whitish false membrane which soon disappears exposing the black, agglutinate, compact spore mass. Spores when mature medium to deep reddish brown, often with smoky tint, globose to oval, occasionally oblong or subangular, sometimes laterally compressed, smooth to indistinctly pitted, or faintly ornamented with short, fine, sinuous striae, 12–17 × 9–15.5 μ, epispore 1–1.5 μ thick.

Material examined: On *Fimbristylis autumnalis* (L.) R. & S. United States: Beaumont, Alabama, in Herb. Berk. 4745, sub *U. axicola* var. (K); Spiro, Oklahoma, in Barthol. Fungi Columb. 4406 (BPI); Virginia, type of *U. fimbristylis* (CH).

On *Fimbristylis diphylla* (Retz.) Vahl. Brazil: Apiahy, Aug. 1888, J. I. Puiggari 2875 (LP); Taquari, Mar. 1940, Costa Nets (LP). Ceylon: Bandarawela, Apr. 2, 1904, T. Petch 2552 (BPI). China: Canton, Kwangtung, C. W. Howard (BPI); Tsingyen ,Kweichow, Aug. 19, 1940, S. C. Liu; Chengtu, Szechwan, Nov. 1947, K. R. Lin; Taipeh, Taiwan, Nov. 12, 1920, H. Sueda, type of *C. suedae.* Dominican Republic: St. Domingo, in Herb. Berk 4745, *type* (K). Indo-China: Tonkin, Feb. 1889, B. Balansa 33, sub *Cintractia junci* Trel. (FH). Paraguay: Cordillera de Peribebuy, Mar. 25, 1883, B. Balansa 3775, type of *U. peribebuyensis* (LP).

On *Fimbristylis* ? *ferruginea* (L.) Vahl. Philippine Islands: Manila, Luzon, Sept. 30, 1945, C. T. Rogerson 687 (BPI).

On *Fimbristylis kagiensis* Hay. China: Taichung, Taiwan, Oct. 11, 1913, Y. Fujikuro, type of *C. fimbristylis-kagiensis.*

On *Fimbristylis monostachaya* (L.) Hassk. Philippine Islands: Manila, Luzon, Sept. 30, 1945, C. T. Rogerson 686 (BPI).

On *Fimbristylis* sp. Cuba: in C. Wright, Fungi Cub. Wright. 604 (FH). Paraguay: in Balansa, Pl. Parag. 4294, sub *Ustilage* *juncicola* Speg. (FH). India: Cocanada, Madras, Dec. 1906, S. Sundararaman, type of *C. peribebuyensis* var. *major* (ND).

On *Fimbristylis tenera* R. & S. India: Pak Pattan, Punjab, Oct. 1936, S. Ahmad 78 (BPI).

In the type collection of this species, the spores are smooth; whereas in certain other collections they may be faintly ornamented by pittings or short striae. On the basis of this character alone, it seems not advisable to split this old and well-known species.

CINTRACTIA CYPERI Clint., Proc. Bost. Nat. Hist. **31**: 400. 1904

Sori in the ovaries, globoid, 1–1.5 mm. diam., more or less exposed between the spreading glumes at later stages; spore mass black, agglutinate and rather compact until maturity. Spores often loosely agglutinated into balls, chiefly polyhedral, 11–21 × 9–16 μ, most elongate ones up to 25.5 μ in length, yellowish brown; epispore 1.5 μ thick, under oil immersion lens appearing as if decorated by very short, sinuous, depressed striae.

Material examined: On *Cyperus filiculmis* Vahl. United States: North Haven, Connecticut, in Seym. & Earle, Econ. Fungi C102, *type* (BPI); Montowese, Connecticut, Sept. 14, 1903, G. P. Clinton (CH); Ocean Gate, New Jersey, Aug. 17, 1919, P. Wilson (BPI).

Zundel (3) recorded *Cyperus cylindricus* and *C. grayii* as the hosts of this species. The latter name is apparently a mistake, since the New Jersey collection actually contains a mixture of healthy plants of *C. grayii* and smutted plants of *C. filiculmis.*

This species is closely related to *Cintractia gigantospora* Liro but differs from it in having chiefly polyhedral and lighter colored spores.

CINTRACTIA FIMBRISTYLICOLA Pavgi & Mundk., Indian Phytopath. 1: 108. 1948

Sori in the ovaries, globoid, 1–1.5 mm. in diameter, black, compact, each at first covered by a thin white false membrane. Spores deep reddish brown, globose to oval, mostly laterally compressed, 8–12 μ in length; epispore approximately 1 μ thick, densely verruculose, often appearing as semi-reticulate under higher magnifications.

Material examined: On *Fimbristylis complanata* Link. India: Chatrapur, Ganjam, Orissa, Aug. 30, 1904, E. J. Butler, *type* (ND).

This species is very similar to *Cintractia fimbristylis-miliaceae* (P. Henn.) Ito and can be distinguished from the latter only by smaller and deeper colored spores. More collections should be studied to ascertain the range of variation in this group of fungi.

CINTRACTIA FIMBRISTYLIS-MILIACEAE (P. Henn.) S. Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 92. 1935

Ustilago fimbristylis-miliaceae P. Henn., in Engl. Bot. Jahrb. **37**: 156. 1905.
Cintractia clintonii Cif., Ann. Myc. **26**: 30. 1928.
Cintractia pulchra S. Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 92. 1935.

Sori in the ovaries, forming globoid, black, compact spore masses, 0.5–1.5 mm. in diameter, each covered at an early stage by a whitish false membrane. Spores when mature yellowish to reddish brown, spherical to oval, occasionally subangular, densely verruculose, 9.5–14.5 μ in length, epispore 0.5–1 μ thick.

Material examined: On *Fimbristylis holwayana* Fernald. Mexico: Guadalajara, Oct. 12, 1903, C. G. Pringle, type of *C. clintonii* (CH).

On *Fimbristylis miliacea* Vahl. Japan: Yukatabara, Iwaki, Kisuta, Sept. 1890, S. Kusano 782, *type* (CH); Mikazuki, Tosa, Oct. 1907, T. Yoshinaga, type of *C. pulchra;* Obi, Oct. 1890, S. Yoshida (FH).

The spores of this species are not smooth as originally described by Hennings. The Mexican collection differs from those from Japan in having a thicker, deeper colored, and less evidently verruculate epispore. The variation may be due to the degree of maturity. The Mexican collection is apparently in an earlier stage of development which is indicated by the presence of numerous, hyaline, agglutinate, immature spores.

CINTRACTIA LIMITATA Clint., Proc. Bost. Soc. Nat. Hist. **31**: 399. 1904. (FIG. 1)

Cintractia axicola (Berk.) Cornu var. *minor* Clint., Jour. Myc. **8**: 143. 1902.
Cintractia togoensis P. Henn., in Engl. Bot. Jahrb. **38**: 119. 1905.
Cintractia congensis P. Henn., Ann. Mus. Congo Bot. V. **2**: 87. 1907.

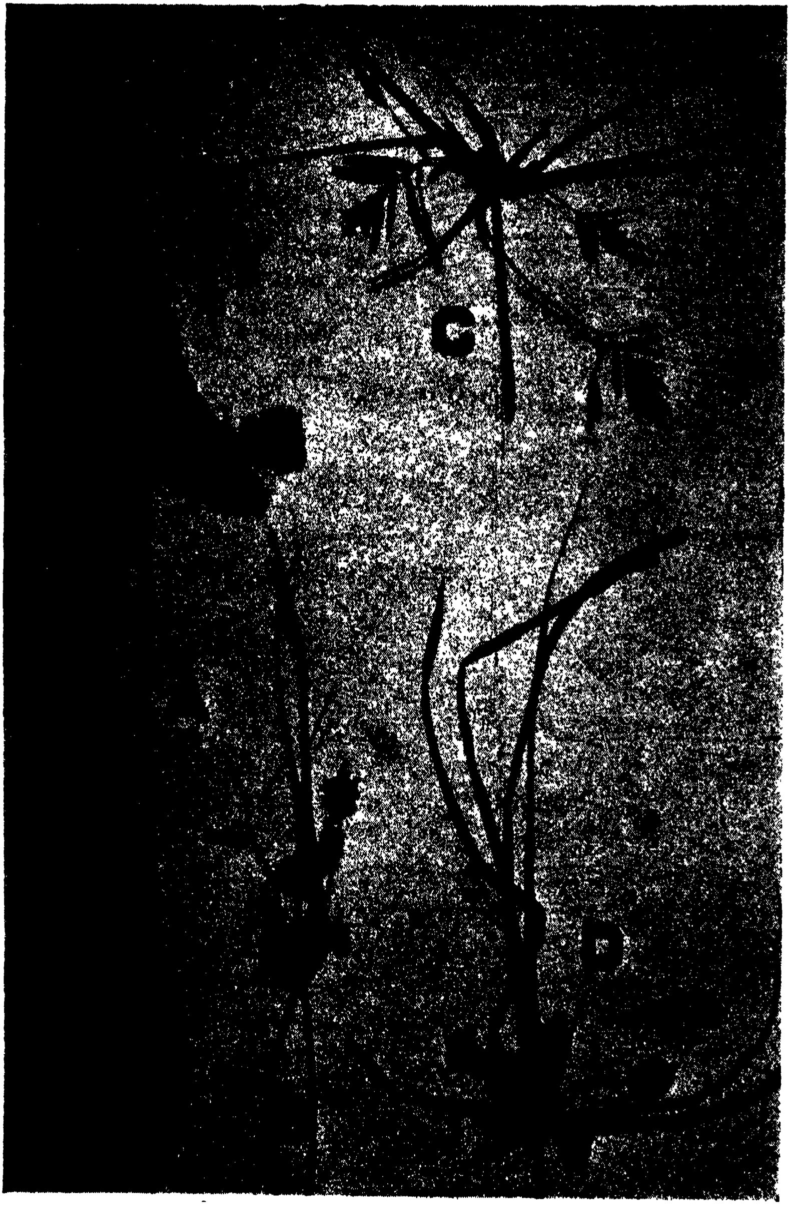

FIG. 1. *Cintractia limitata.*

Cintractia cyperi-polystachyi P. Henn., Philipp. Jour. Sci. C. **3**: 41. 1908.
Cintractia minor Jacks., Mycologia **12**: 153. 1920.
Ustilago chacoensis Hirschh., Not. Mus. La Plata **6**: 479. 1941.
Ustilago mariscana Zundel, Mycologia **35**: 165. 1943.
Cintractia distans Mundk., Indian Jour. Agr. Sci. **14**: 50. 1944.

Sori at the base of primary or auxiliary rays, cylindrical, forming black, agglutinate spore masses surrounding the branches, extending up to 1 cm. in length and 7 mm. in diameter, each covered at first by a whitish false membrane which soon disintegrates; often also infecting the ovaries, each forming a globoid black spore mass surrounding a rudimentary columella, 1–2 mm. in diameter, agglutinate at earlier stages, becoming rather dusty, exposed between the spreading glumes, and often spreading to adhere to the glumes and other floral parts. Spores when mature ochraceous brown to deep reddish brown, often with smoky tint, spherical to oval, occasionally subangular, frequently laterally compressed, 9–14.5 × 7.5–13 μ; epispore smooth, 1 μ or less thick.

Material examined: On *Cyperus compressus* L. China: Taitung, Taiwan, May 21, 1911, K. Sawada.

On *Cyperus corymbosus* Rottb. Ceylon: Peradeniya, June 29, 1913, T. Petch 3719.

On *Cyperus cyperoides* (L.) O. Ktze. Gold Coast: Abori, May 12, 1925, R. H. Bunting (CMI). Nigeria: J. West 25 (CMI). Sierra Leone: Newton, June 26, 1935, F. C. Deighton (CMI). Uganda: Serere Teso, June 29, 1939, C. G. Hansford 1602 (CMI). Union of South Africa: Imfulazane, Melmoth, Natal, Dec. 1, 1919, A. O. D. Mogg, type of *U. mariscana* (BPI).

On *Cyperus digitatus* Roxb. China: Taichung, Taiwan, July 2, 1936, Y. Hashioka.

On *Cyperus distans* L. f. Belgian Congo: Kinshasa, June 1, 1916, H. Vanderyst (BR); prov. Orientale, Apr. 1926, J. Ghesquière 562 (BR). India: Dacca, Bengal, Aug. 1940, P. Maheshwari, type of *C. distans* (ND).

On *Cyperus dubius* Rottb. Uganda: Serere Teso, June 29, 1939, C. G. Hansford 1644 (CMI).

On *Cyperus esculentus* L. Belgian Congo: Kelomila, May 1906, H. Vanderyst 105, type of *C. congensis* (BR); Boma, Apr. 1913, H. Vanderyst 1299 (BR).

On *Cyperus grayii* Torr. United States: Vineland, New Jersey, July 9, 1912, W. R. Jones (BPI); Sandy Hook, New York, in

Ell. & Ev. N. Amer. Fungi 2423, type of *C. axicola* var. *minor* (BPI).

On *Cyperus ligularis* L. Puerto Rico: Mayaguez, Apr. 23, 1904, G. P. Clinton, *type* (CH). Venezuela: Ocumare de la Costa, Mar. 19, 1938, C. E. Chardon 2489 (BPI).

On *Cyperus longus* L. Equatorial Africa: Gabun, June 1908, J. Mildbraed (CH).

On *Cyperus macrocarpus* (Kunth.) Boeck. var. *pseudoflavus* (K. Schum.) Kuekenth. Belgian Congo: Kinsatu, May 5, 1906, H. Vanderyst 32 (BR). West Africa: Lome, Togo, Camerun, Apr. 1900, Warnecke 118, type of *C. togoensis* (CH).

On *Cyperus malaccensis* Roxb. China: Taipeh, Taiwan, Sept. 27, 1917, Y. Fujikuro. Philippine Islands: Aparri, Cagayan, Luzon, Jan. 8, 1924, M. S. Clemens (BPI).

On *Cyperus ornans* Suring. Gold Coast: Anjinam, June 22, 1937, F. C. Deighton (CMI).

On *Cyperus polystachyus* Rottb. Philippine Islands: Manila, Aug. 1906, E. D. Merrill 5195, type of *C. cyperi-polystachyi.*

On *Cyperus rotundus* L. Anglo-Egyptian Sudan: Kadugli, Oct. 1947, J. Tarr (CMI). Argentina: Chaco, May, 1940, C. Carrera, type of *U. chacoensis* (LP).

On *Cyperus* sp. Brazil: Restinga de Cabo Frio, Rio de Janeiro, Oct. 16, 1938, A. P. Viégas & H. P. Krug (BPI).

On *Cyperus sphacelatus* Rottb. Nigeria: J. West 39 (CMI). Puerto Rico: Humacao Playa, H. H. Whetzel *et al.* 3038 (BPI); Manati, Jan. 25, 1938, W. A. McCubbin (BPI).

On *Cyperus tuberosus* Rottb. India: Lahore, Punjab, 1938, H. Chaudhari (CMI).

On *Cyperus zollingeri* Steud. Sierre Leone: Kichom, N. prov., June 1, 1928, F. C. Deighton (CMI).

The names commonly applied to this fungus are *C. peribebuyensis, C. minor* and *C. limitata.* As indicated in a previous note (2), the first is a synonym of *C. axicola.* The latter two were supposed to be distinguished by the location of sori, *C. minor* being confined to the base of the rays and *C. limitata* to the spikelets. However, even in the type collection of *C. minor* (FIG. 1, B), as mentioned by Clinton (1) himself, the infection also occurs in the floral organs forming a conspicuous smutty mass. It is also not uncommon to find in the collections labelled as *C. limitata* that both the spikelets and the base of the rays are smutted by the same fungus. Figure 1 A, illustrates such a case in a collection from Venezuela on *Cyperus ligularis*, the type host of *C. limitata*. Therefore there is no real distinction between these two species in the characters of both the sori and the spores. According to the International Rules of Botanical Nomenclature, *C. limitata* is the valid name.

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EXPLANATION OF FIGURE

FIG. 1. *Cintractia limitata*. A. On *Cyperus ligularis*, showing infection in the spikelets and at the base of the ray (Venezuela, C. E. Chardon 2489). B. On *Cyperus grayii*, showing infection in the spikelets and at the base of the rays (type of *Cintractia axicola* var. *minor*). C. On *Cyperus polystachyus* (type of *Cintractia cyperi-polystachyi*). D. On *Cyperus macrocarpus* var. *pseudoflavus* (Congo, H. Vanderyst 32, sub *Cintractia togoensis*). All × 1.

A NOTE ON THE CULTURE OF DIPODASCUS UNINUCLEATUS IN DEFINED MEDIA *

Eugene L. Dulaney and F. H. Grutter

During a recent survey of fungi for the production of vitamins, it was found that *Dipodascus uninucleatus* failed to grow in certain media. Good growth, however, was obtained in a medium containing 5.0 grams of glucose and 10.0 grams of Difco yeast extract per liter of distilled water. When three-day old mycelium grown in this glucose-yeast extract broth was washed with sterile distilled water and used to inoculate a vitamin free medium, only sparse growth resulted. The mycelium constituting the sparse growth in the vitamin free medium was likewise washed and used to inoculate other flasks of the vitamin free medium. No visible growth occurred. The vitamin free medium, which will be referred to hereafter as the basal medium, contained the following constituents per liter of distilled water: glucose 10.0 g., vitamin free acid hydrolyzed casein 10.0 g., K_2HPO_4 2.0 g., $MgSO_4 \cdot 7H_2O$ 1.0 g., NaCl 0.2 g., $CaCl_2$ 0.2 g., $FeSO_4 \cdot 7H_2O$ 0.005 g. and $ZnSO_4 \cdot 7H_2O$ 0.005 g.

The results noted above indicate that *Dipodascus uninucleatus* requires one or more factors found in yeast extract for its growth. These possible nutritional deficiencies were investigated using the following experimental conditions. The media were dispensed in 25 ml. amounts in 125 ml. Erlenmeyer flasks which were plugged with non-absorbent cotton and sterilized by autoclaving at 120 lbs. for 17 minutes. All glassware was acid cleaned and thoroughly rinsed in distilled water before using. A master inoculum was prepared as follows and used for all experiments. Thoroughly washed mycelium grown in glucose-yeast extract broth was used to inoculate four flasks containing 25 ml. of the basal medium. After 72 hrs. incubation at 28° C on a rotary shaker, the sparse growth was removed from the flasks by centrifugation, pooled, washed three times with sterile distilled water and made up to a final volume of approximately 45 ml. with sterile distilled water. This washed suspension was stored at 4° C for several weeks and used at intervals. Two drops of this washed suspension were used to inoculate each flask. Each treatment was run in triplicate. All experiments were incubated at 28° C on rotary shakers moving at 220 r.p.m. so that they described a horizontal circle one and one-half inches in diameter.

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* Contribution from the research laboratories of Merck & Co., Inc., Rahway, N. J.

It seemed probable that the required factors in the yeast extract were vitamins or nucleic acid constituents or both. This possibility was investigated by supplementing the basal medium with a vitamin mixture and nucleic acids singly and in combination. The results obtained after three days incubation are shown in table I.

TABLE I

A VITAMIN MIXTURE AND NUCLEIC ACIDS AS SUPPLEMENTS TO THE BASAL MEDIUM

Treatment	Dry wt. mg./ml.	pH
Basal Medium	No growth	
B.M. plus vitamin mixture [1]	5.86	3.4
B.M. plus nucleic acids [2]	0.66	3.6
B.M. plus vitamin mixture [1] and nucleic acids [2]	5.56	3.1

[1] One ml. of vitamin mixture was added to 24 ml. of the basal medium. The final concentration of the different vitamins in each flask was as follows: riboflavin 1.0 μg./ml., i-inositol 0.1 mg./ml., biotin 0.04 μg./ml., vitamin B_1 0.1 μg./ml., glutamine 5.0 μg./ml., pantothenic acid 1.0 μg./ml., vitamin B_{12} 0.001 μg./ml., nicotinic acid 10.0 μg./ml., pyridoxine 10 μg./ml., choline 5.0 μg./ml., para aminobenzoic acid 10 μg./ml., and folic acid 0.1 μg./ml.

[2] 12.5 mg. of yeast nucleic acid and 12.5 mg. of thymus nucleic acid were added directly to each flask.

No growth was obtained in the basal medium whereas good growth occurred in the medium supplemented with the vitamin mixture. The growth in the nucleic acid supplemented medium was probably due to vitamin contaminants in the nucleic acids. The addition of both nucleic acids and the vitamin mixture did not increase the amount of cell weight over that obtained in the medium supplemented with vitamins alone.

In order to determine what specific vitamins were required, the same vitamins that had been used in the mixture were divided into groups and used to supplement the basal medium. Growth occurred only in those treatments to which both vitamin B_1 and biotin were added. The effects of supplementing the basal medium with biotin, vitamin B_1 and i-inositol singly and in combination are shown in table II. None of the vitamins alone stimulated growth.

TABLE II

VITAMINS USED SINGLY AND IN COMBINATION TO SUPPLEMENT THE BASAL MEDIUM

Treatment [1]	Dry wt. mg./ml.
Biotin	No growth
Vitamin B_1	No growth
I-inositol	No growth
Biotin and vitamin B_1	4.9
Vitamin B_1 and i-inositol	0.57
Biotin and i-inositol	No growth
Vitamin B_1, biotin, and i-inositol	5.1
Vitamin mixture	5.2

[1] One ml. of each vitamin solution was added to enough medium in each flask so that the final volume in each flask was 25 ml. This resulted in some slight variation in the concentration of medium constituents in those flasks to which more than one ml. of vitamin solution was added. The final concentration of each vitamin, however, was the same as the concentration of the same vitamin in the flasks containing the vitamin mixture.

There was some slight growth in the medium containing vitamin B_1 and i-inositol, and no growth in the medium containing biotin and i-inositol. Biotin and vitamin B_1, with or without i-inositol, replaced the vitamin mixture. No measurements have been made of the amount of growth that occurs in the glucose-yeast extract medium.

Dipodascus uninucleatus requires no exogenous supply of organic nitrogen. Three compounds were substituted for the hydrolyzed casein in an experiment, the results of which are shown in table III. Urea and nitrate nitrogen did not serve as sole nitrogen sources. Ammonium nitrogen, however, was readily available.

TABLE III

SUBSTITUTION OF NITROGEN COMPOUNDS FOR THE CASEIN HYDROLYSATE

Treatment [1]	Dry wt. mg./ml.	pH
$(NH^4)_2HPO^4$	4.67	2.5
$NaNO^3$	No growth	
Urea	No growth	
Casein hydrolysate	4.43	3.1

[1] The casein hydrolysate was used at a concentration of 10 mg./ml. The other nitrogen compounds were used at a level of 5 mg./ml. All treatments contained 0.1 μg./ml. of vitamin B_1 and 0.04 μg./ml. of biotin.

These results indicate that *Dipodascus uninucleatus* requires an exogenous source of biotin and vitamin B_1. The possibility exists, however, that these deficiencies are peculiar to our strain and that, as a rule, the genus *Dipodascus* is not heterotrophic. Further investigation of other isolates is needed to establish this.

The drop in pH of the culture media in these experiments indicates the accumulation of one or more organic acids.

A NEW ACHLYA FROM FLORIDA[1]

A. W. Ziegler[2]

(WITH 15 FIGURES)

A water collection from a swamp in Wakulla County, Florida, 15 miles south of Tallahassee, Florida, has yielded an undescribed species of *Achlya.*

Achlya tuberculata n. sp.

Mycelium densum; hyphae usque ad 1 cm. longae in seminibus *Cannabis sativae;* cultura circa 1.5–2.5 cm. in diametrum. Sporangia copiosa in culturis juvenilibus, longa, cylindrica, 162–448 μ longa et 10–15 μ lata. Ejectio sporarum typica generis. Gemmae copiosae praesertim in culturis natu majoribus, valde variae. Oogonia globosa aut aliquando clavata, 44.4–92.5 μ in diametrum, ornata projectionibus obtusis, 1.2–3.7 μ longis; tunica tenuis in apice projectionis. Longitudo caulis oogonii 21.5–184 μ. Ova eccentrica, plerumque 3, globosa aut aliquando ovoidea, plurimum ferme 24.1 μ in diametrum, non complentes oogonium. Dimidia ovorum auctorum in aqua distillata deficit ante maturitatem; in decoctione foliorum *Quercus* ferme omnia ova maturescunt. Antheridia rara, semper diclina, reperta praecipue in culturis juvenilibus in aqua distillata aut cum aut sine foliis *Quercus.*

Mycelial growth dense, hyphae reaching a length of one-half to 1 cm. on hemp seed in charcoal filtered distilled water, diameter of the culture about 1.5–2.5 cm. Sporangia plentifully formed in young cultures, long, cylindrical, ranging from 162 by 10 μ to 448 by 15 μ. Spore discharge typical for the genus. The cluster of spores usually falls to the bottom of the dish soon after formation and gradually breaks apart into individual spores. Gemmae plentiful especially in older cultures, consisting of a row of swollen or non-swollen hyphal segments, single enlarged spherical or reniform bodies, or smaller spherical cells in a short chain. Gemmae germinate by a germ tube producing spores in the manner of the genus. Oogonia spherical or occasionally clavate with blunt pro-

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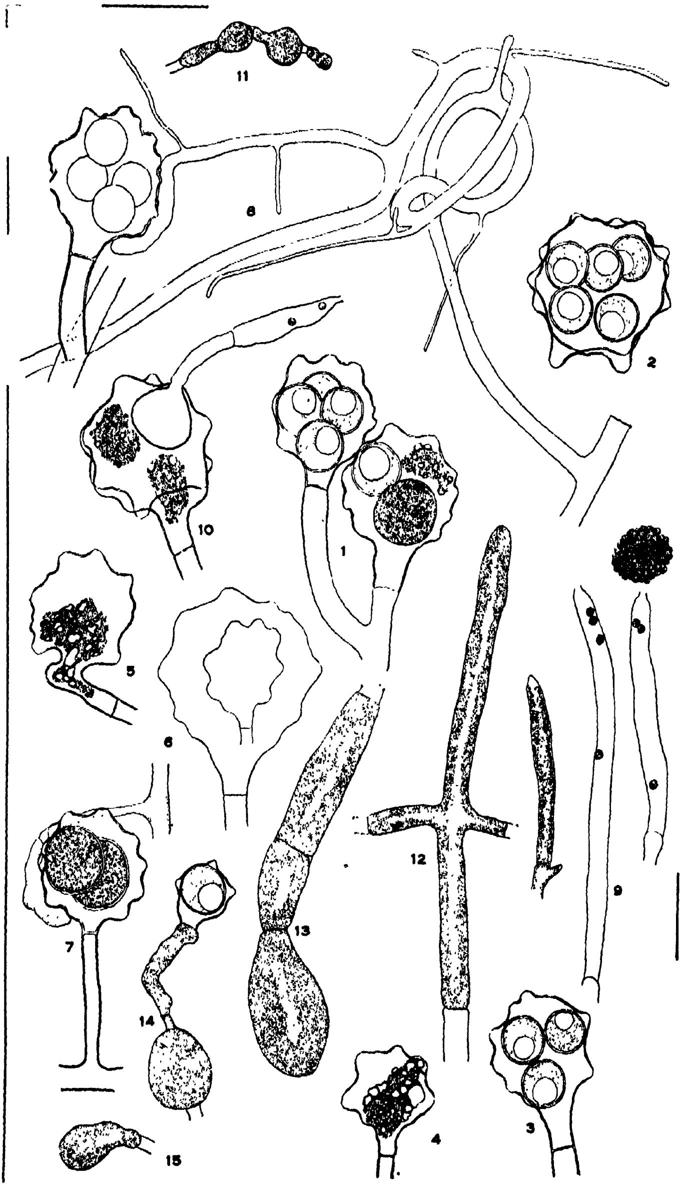
[2] Assistant Professor of Botany, Florida State University. The writer is indebted to Dr. J. N. Couch for his advice and aid and to Mrs. C. D. Beers for the Latin description.



FIGS. 1–15. *Achlya tuberculata.*

jections and varying in size without the projections from 44.4 μ to 92.5 μ in diameter. The end wall of the projections is usually thinned. Projections vary from 1.2 μ to 3.7 μ in length. Length of oogonial stalk from 21.5 μ to 184.0 μ. Eggs eccentric, 1–6, mostly 3, ranging from 18.5 to 33.3 μ in diameter, mostly about 24.1 μ not filling the oogonium completely, usually spherical but sometimes ovoid through pressure. When grown in distilled water more than one-half of the eggs formed disintegrate before maturity even though an antheridium may be applied to the oogonial wall. However, if grown in distilled water to which oak leaves have been added 94–99% of the eggs reach maturity. Antheridia scantily formed and occurring mainly in young cultures in water with or without the addition of oak leaves. Antheridia always diclinous and those that appear to function become empty before the eggs are mature. Antheridial tube seen only twice. Mature eggs germinate after 2 months by short germ tubes with apical sporangia.

In water on hemp seed bait. Collected by the author in a swamp in Wakulla County, Florida, 15 miles south of Tallahassee, Florida, July 9, 1949.

On first seeing this fungus, the author classified it as *A. crenulata* Ziegler. However, later observations show that it differs in several ways. The oogonia of *A. tuberculata* show a tremendous size variation which is not characteristic of *A. crenulata*. The length of the oogonial stalk is highly variable in *A. tuberculata* whereas that of *A. crenulata* is about equal to the width of the oogonium. There is also a slight difference in the usual egg size between the two forms and the gemmae of *A. tuberculata* are wholly unlike those of *A. crenulata* (4).

One of the peculiar characteristics of this form is its disintegrating eggs which occur when it is grown in charcoal filtered distilled water in Petri dishes. The average of four separate counts of 100 oogonia each from four cultures of varying ages showed that 59% of the oogonia contained distintegrated eggs entirely. Of the remaining 41%, 28% of the oogonia contained eggs all of which developed to maturity, and 13% contained both mature and disintegrated eggs. No parasite has been found to be present and tests have been made which proved that the cultures were bacteria free. However, if the form is placed in an oak leaf decoction made by boiling a few dry oak leaves in distilled water for 10 minutes the egg disintegration is considerably lessened. In this procedure, 94–99% of the oogonia formed mature eggs. Antheridial growth is only very slightly stimulated by this treatment. The characteristic discussed above is not a feature of the growth of *A. crenulata.* Experiments involving solutions of known pH and content, and the effect of returning the form to its natural habitat are now being performed by Miss Betty Linthicum and the author.

A. tuberculata may be distinguished from *A. subterranea* Coker and Braxton by the size of its oogonia and the usually androgynous antheridium of *A. subterranea* (2). It differs from *A. recurva* Cornu in the bluntness of its spines and in its diclinous antheridia (3) and from *A. radiosa* Maurizio especially in the number of eggs produced (1). The new form can easily be separated from *A. abortiva* Coker and Braxton by its spherical oogonia, pitted oogonial walls, and smaller eggs (2). The eccentric eggs and diclinous antheridia of *A. tuberculata* distinguish it from *A. colorata* Pringsheim (1).

It may be of interest to note that this fungus was collected in a water sample from a cypress swamp whose waters were exposed to direct sunlight all day. The author failed to take the temperature of the water but it felt like a very warm bath to the hand. *A. tuberculata* is grown in the laboratory on maltose peptone agar but is very slow in its growth rate.

Gemma formation apparently follows no set pattern in this species. The gemmae are often irregularly shaped and bizarre in form. A vegetative hypha often forms a row of swollen or non-swollen cells with the apical one either rounded or pointed. The cytoplasm of the oogonial stalk occasionally produces an elongated or spherical gemma (FIG. 14). Large spherical or kidney shaped gemmae are often seen as well as short chains of cells strung together like beads. Numerous variations of the above patterns also occur. However formed, they germinate by short germ tubes and spores.

A. tuberculata germinated readily when the mature eggs were about two months old. The eggs were placed in distilled water filtered through charcoal and completed their germination in about 32 hours. The details of the germination process closely follow those described for *A. flagellata* Coker. This species formed a short germ tube with an apical sporangium and therefore falls into type A of the patterns of germinations found in the Saprolegniaceae (5).

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EXPLANATION OF FIGURES

FIG. 1. Two oogonia with mature and disintegrating eggs, × 750. FIGS. 2–3. Oogonia with mature eccentric eggs, × 750. FIGS. 4–5. Oogonia with disintegrated eggs, × 750. FIG. 6. Outline drawing of 2 oogonia showing great variation in size, × 750. FIG. 7. Oogonium with diclinous antheridium and immature eggs, × 750. FIG. 8. Outline drawing of oogonium with coiled diclinous antheridial branch, × 750. FIG. 9. Typical sporangia, × 350. FIG. 10. Oogonium containing several disintegrated eggs and one which has germinated and produced a germ tube with an apical sporangium, × 750. FIG. 11. A gemma, × 350. FIGS. 12–13. Gemmae, × 750. FIG. 14. Gemmae formed from the oogonial stalk, × 350. FIG. 15. Gemma, × 350.

A NEW RUST ON DESCHAMPSIA[1]

D. B. O. Savile[2]

Aecidium Graebnerianum P. Henn. was described from a specimen on *Habenaria dilatata* from Death Valley, Calif. The species has since been recorded on *H. borealis, H. bracteata, H. gracilis, H. hyperborea, H. leucostachys* and *Orchis aristata*, in California, Oregon, Washington, Montana, British Columbia and especially, Alaska (Arthur, 1), but it has apparently never been reported east of the Rocky Mts. In 1949, aecia apparently indistinguishable from it were found at two stations in Quebec. Mr. A. Payette collected a rust on *Habenaria psychodes* at Rivière Ouelle, on the south shore of the Gulf of St. Lawrence, about 70 miles below Quebec City. The aecia in this specimen (DAOM 23448), collected on 20 July, were largely spent, but sufficient spores were available to show that it agreed well with Barth. N. A. Ured. 1401, on *H. gracilis* from Glacier, B. C.

On the same date that Mr. Payette made his collection, the writer found a similar *Aecidium* at Great Whale River, on the east coast of Hudson Bay, on *H. dilatata* (DAOM 23447). Owing to the late spring at this station, some aecia were shedding spores at this time and others had not yet opened. Fortunately, this colony was readily accessible and a careful search showed that no other *Aecidium* occurred within fifty yards. Several infected plants were accordingly left for future observation in the hope that the rust would complete its life history. *H. dilatata* grows in very wet ground, often along streams or in shallow springs, although some of the hosts of *Aecidium Graebnerianum* occur in somewhat drier situations. The rusted colony was on a narrow strip of low, springy ground between a lake and a low cliff. *Carex* spp. formed about half the herbaceous cover and seemed to be the most likely alternate hosts.

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[2] Associate Plant Pathologist.

With the progress of the season, it became evident from observations on other species that the cool, short growing season permitted the survival of heteroecious Pucciniaceae (other than species, such as *Puccinia Poae-sudeticae,* that overwinter in the uredinial state) only if the alternate hosts are virtually contiguous. As time passed with no sign of infection on the sedges, it was feared that the telial host might have been adjacent only to plants that had been collected for the aecial specimen. Eventually, in mid August, a minute scrutiny of all the surrounding vegetation revealed a number of very small pustules, identifiable as such only under the hand lens, on a few plants of *Deschampsia atropurpurea.* Most of the pustules were on vegetative offsets, but a trace of infection occurred on one fruiting plant and allowed confirmation of the identity of the grass host. Only a few plants of *D. atropurpurea* were present and none were found elsewhere; nor was the *Aecidium* seen in any of many other colonies of *Habenaria dilatata, H. hyperborea,* or *H. obtusata* that were examined. The grass rust was heavy only on leaves within a few inches of *H. bracteata* and none occurred on plants three to five feet distant. The rust was left until 22 August, to allow further development of some of the immature sori, but was then collected for fear that all uredinia might be displaced by telia.

When microscopic examination was finally possible, the rust proved to be a species of *Puccinia* with teliospores possessing apical processes similar to those of *P. coronata.* In fact, the teliospores differed from those of *P. coronata* only in being distinctly shorter than the shortest forms of that variable species. The urediniospores also had appreciably thinner walls than is usual in *P. coronata.* The germ pores were obscure, partly, no doubt, because the walls were thin and only slightly pigmented. It is thus not known whether the number and disposition of the pores is different from that in *P. coronata* in which the pores are commonly about 6 to 8, scattered and often indistinct.

It must be emphasized that there is no possibility of this rust being *Puccinia coronata* in any accepted definition of that species. No *Rhamnus* or *Elaeagnus* occurs within many miles of the location. *Shepherdia canadensis* does occur, but it is restricted to the immediate coast and the nearest plants were some two miles distant. Furthermore, the correlation of the rust with proximity to the *Aecidium* was so striking as to preclude any doubt of the connection between the two stages.

An examination of the late W. P. Fraser's collections on *Deschampsia caespitosa* from Alberta and Saskatchewan, assigned by him to *Puccinia coronata,* showed that they are definitely that species; they have thicker urediniospore walls and much longer teliospores than the rust on *D. atropurpurea.* Barth. N. A. Ured. 431, on the same host from Wyoming, consists only of uredinia, but the spores are thick-walled and similar to those in Fraser's collections. These specimens apparently belong to some variety of *P. coronata* other than those recognized by Fraser and Ledingham (2).

A study of *Puccinia coronata* on one or more species each of *Agropyron, Agrostis, Avena, Beckmannia, Bromus, Calamogrostis, Festuca, Holcus, Lolium, Phleum, Poa* and *Scolochloa* gave a range of urediniospore wall thicknesses of 1.0–2.0 μ, except on *Agropyron* spp. in which it was 0.7–1.4 μ. The total range of teliospore lengths in these specimens was 33–78 μ with the exception of a collection on *Agropyron tenerum* in which the range was 30–70 μ. The measurements given below for the rust on *Deschampsia atropurpurea* show small but definite differences in these two characters, which, with the occurrence of the aecia on a monocotyledonous host, make it advisable to treat it as a distinct species. It is a pleasure to name this rust for Mr. I. L. Conners, of this Division, whose extensive knowledge of the graminicolous rusts has long been of great value to Canadian mycologists and plant pathologists.

Puccinia Connersii sp. nov.

Pycnia ignota. Aecia hypophylla, rarius epiphylla. Aeciosporae 16.0–23.0 × 12.5–19.0 μ; episporio hyalino, 0.5–1.2 μ crass., minute verrucoso. Celluli peridii 18–45 × 15–26 μ, pariete externo minute striato vel verrucoso, interno verrucis c. 3.0–3.5 μ alt. × 0.3–1.2 μ lat. obtecto. Uredinia et telia minuta, amphigena. Urediniosporae 17.5–21.5 × 15.5–20.5 μ; episporio pallide flavo, 0.5–1.2 μ crass., leniter echinulato, poris indetectis. Telia epidermide tecta, compacta. Teliosporae (23)25–36(39) × 13–20 μ, summae digitate appendiculatae; appendiculis 1.0–6.0 μ long., (0)3–4(8) in teliospora; episporio baso ochraceo 0.5–1.0 μ crass., summo castaneo 1.5–3.0 μ crass. appendiculis exclusis.

Aecia in foliis *Habenariae dilatatae.* Uredinia et telia in foliis *Deschampsiae atropurpureae,* prope Flumen Magnum Balaenarum, Canada.

Type. On *Deschampsia atropurpurea* (DAOM 23446), Great Whale River, Quebec, Canada, 22 Aug. 1949, in the Mycological Herbarium, Division of Botany and Plant Pathology, Ottawa, Canada; a portion of the collection in the Arthur Herbarium, Purdue University.

The type of *Aecidium Graebnerianum* has not been seen, but from material and descriptions available it is probably identical with *P. Connersii.*

There is no positive way of forecasting what other grasses will prove to be telial hosts for this species. If, as seems probable, all the collections ascribed to *Aecidium Graebnerianum* are the aecial stage of *P. Connersii,* there may be several grass hosts; but a consideration of the North American species of *Deschampsia* shows that they cover the range and habitats of the rusted orchids fairly completely, and there may be no other genus of grasses involved. *D. danthonioides* and *D. elongata* occur, according to Hitchcock (**3**), in open ground throughout the western range of *Ae. Graebnerianum. D. atropurpurea* occupies open or wooded, wet ground in the same region, across northern Canada, and southward on mountains in the east to New Hampshire; it does not occur at sea level in southeastern Quebec, but *D. caespitosa* var. *intercotidialis* is common along the shores of the Gulf of St. Lawrence in the vicinity of Rivière Ouelle and will probably prove to be the grass host for the rust in that region. *D. caespitosa* in its various forms covers most of the northern half of the continent and may be a host in some other localities; but, as already shown, the rust known on this species in Alberta, Saskatchewan, and Wyoming is *Puccinia coronata.* Although *P. Connersii* is probably not common in any part of its range, its apparent scarcity must be partly attributed to its inconspicuous aecia and telia.

An interesting feature of *P. Connersii,* and one that further supports its identity with *Ae. Graebnerianum,* is its apparent lack of pycnia. The immaturity of some of the groups of aecia in DAOM 23447 made this collection suitable for the study of pycnia, but no trace of any could be discerned.

ACKNOWLEDGMENTS

The writer wishes to acknowledge the advice of Dr. Geo. B. Cummins on the disposition and affinities of this species. His thanks are also due to Dr. R. C. Russell, who made available for study Prof. W. P. Fraser's collections of *Puccinia coronata* on *Deschampsia caespitosa* from the herbarium of the University of Saskatchewan.

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HISTOPLASMA AND BRAZILIAN BLASTOMYCES *

ELEANOR SILVER DOWDING †

(WITH 6 FIGURES)

Histoplasma capsulatum Darling is the causal organism of human histoplasmosis; *Blastomyces brasiliensis* (Splendore) Conant and Howell, of South American blastomycosis. *Histoplasma* is the more widely distributed, while *B. brasiliensis* is localized—chiefly in Brazil (FIG. 1). There is no suggestion in the literature that they are closely related. They can, in fact, be distinguished one from the other in two ways.

First, in culture, the spores of *H. capsulatum* are more plentiful and are distinctively shaped, while those of *B. brasiliensis* are rarer and lack the distinctive irregular outline of *Histoplasma.*

Second, within the host cell, *Histoplasma* exists as a small oval cell 1.5 μ long, sometimes with a single bud. *B. brasiliensis* is a larger spherical cell 15–20 μ in diameter, surrounded by a number of buds.

However, in cultures of both fungi, spores may be lacking. Upon one occasion in this laboratory there were six cultures of a single strain of *H. capsulatum* on Sabouraud's medium. Two of them were one month old, two, three months, and the remaining two, four months, all growing vigorously. Only one of the six was producing spores. The other five possessed little to distinguish them from *B. brasiliensis.* In some cultures, the writer has seen microconidia of *H. capsulatum* in great abundance, but Howell (9), in his study, discovered so few that he considered them "obviously depauperate structures."

Again, Almeida studied the multiple-budding forms of *B. brasiliensis* as they occurred in animal tissue (1). He pointed out that

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* This investigation was made possible by financial assistance from the National Research Council of Canada and the Dominion Department of Health.

† Mrs. E. S. Keeping.

the uppermost and lowermost slices of serial sections show groups of minute daughter cells separated from the parent cell. His photograph of these bodies within animal tissue strongly recalls *Histoplasma.* Only by searching in other sections would one find the larger cell to which they are attached. Then again, it has recently been shown that the spores of *Histoplasma* may reproduce by multiple budding (8). In short, the writer suggests that the two fungi are similar enough for medical mycologists, under certain conditions, to confuse them, and that a comparative study of their saprophytic phases would be in order.

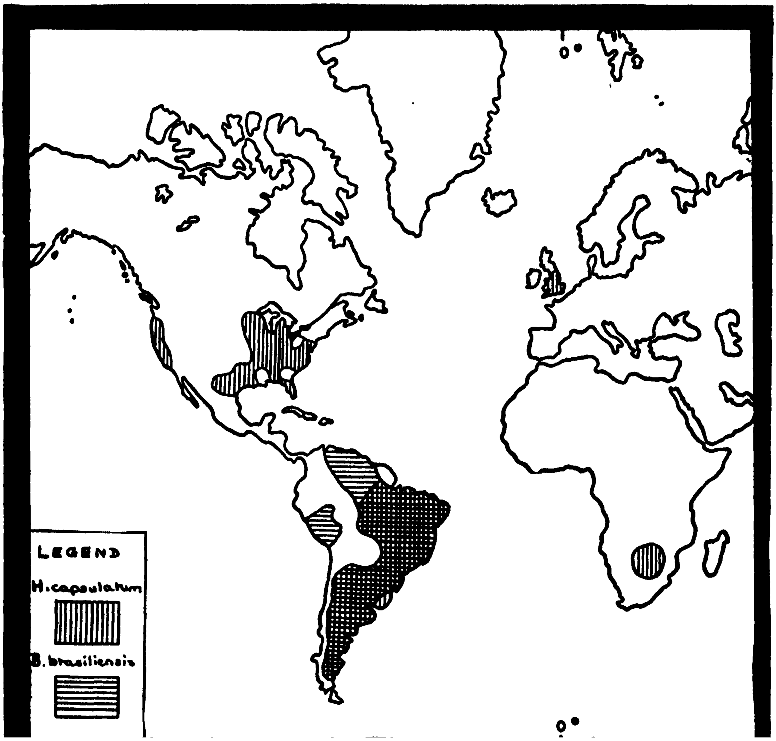

FIG. 1. Distribution of mycoses caused by *H. capsulatum* and *B. brasiliensis.* Adapted from Conant *et al.* "Manual of Clinical Mycology."

Observations on *Histoplasma* have recently been published (8). Some of the results together with a discussion and interpretation of them are given below, followed by a description of *B. brasiliensis* which confirms and extends Almeida's study (1).

MATERIALS AND METHODS

Six strains of *Histoplasma* and seven of *B. brasiliensis,* including *B. cerebriformis* were examined. Those studied in greatest detail were: *H. capsulatum* obtained from Dr. C. W. Emmons of the United States Public Health Service; *B. brasiliensis* from Dr. N. F. Conant of Duke University, and *B. brasiliensis* strain 630 from the National Collection of Type Cultures in Kew, England. A fungus sent for identification by Dr. K. B. Raper of the U. S. Dept. of Agriculture, was classified as *Sepedonium chrysospermum* Fr. and used for comparative study.

H. capsulatum was cultured on Sabouraud's medium, rice as prepared by Conant (3), Littman's agar, malt agar, dextrose broth, cysteine broth (13), and beef broth. *B. brasiliensis* was grown on Sabouraud's medium, plain agar, rice, cornmeal agar, malt agar, soil agar, soil, asparagin synthetic medium, and coffee agar (for the reason that South American blastomycosis is common among coffee workers). Of these substances, the most satisfactory for growth of *Histoplasma* was on Sabouraud's medium. *B. brasiliensis* grew well on Sabouraud's medium, plain agar, and rice, the best sporulation and most characteristic growth being on rice.

HISTOPLASMA CAPSULATUM

Histoplasma capsulatum grows at room temperature with a dense, white, fine, cottony, aerial mycelium sometimes bearing spores. The spores are of two kinds, microconidia and larger spores described in the literature as chlamydospores. The *microconidia,* which are borne laterally, are sessile, pyriform, frequently echinulate, and about 2.5 μ in diameter.

Since the *Histoplasma* "chlamydospore" is neither thick-walled nor resting, the term *macroconidium* is preferable for it, and will be used here. The macroconidia are about five times larger than the microconidia, spherical or pyriform, smooth or somewhat warty at first,[1] later, on Sabouraud's medium, becoming tuberculate with radial projections, and taking on an amber color. A mucilaginous secretion exudes from the pits of these spores and envelops each of them.

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[1] Mature macroconidia remain smooth on malt agar, Littman's agar, dextrose broth and cysteine broth.

The tuberculations of the macroconidia are characteristic and give the spores an appearance rather like *Chenopodium* pollen. Their shape, which may be obscured by the surrounding secretion, is pyriform, and their length about 2.5 μ. Howell (9) has pointed out that macroconidia of *Histoplasma* and the spores of *Sepedonium chrysospermum* both have finger-like projections. In the young spore of both they are hollow and contain protoplasm. However, the writer is convinced that they differ in that the *Sepedonium* spore-projections upon maturity become solid with wall-substance while those of *Histoplasma* remain hollow.

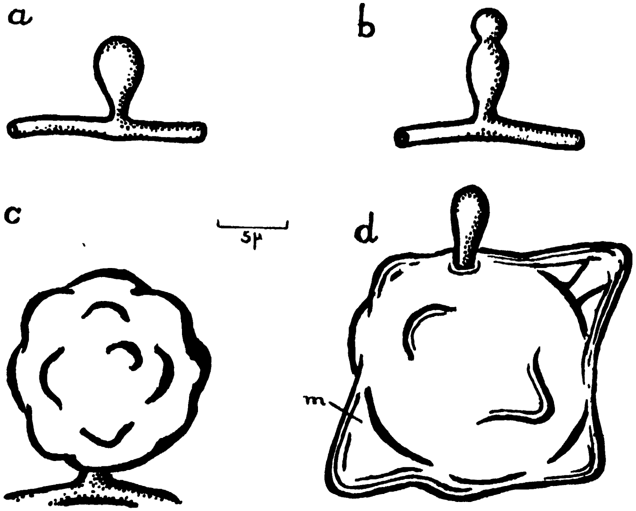

FIG. 2. The spores of *H. capsulatum*: *a* and *b*, microconidia; *c* and *d*, macroconidia; *a* and *c*, primary spores; *b* and *d*, secondary spores; *m*, mucilaginous secretion.

Morphological and cultural studies have shown that the conidial tuberculations are spores (8) (FIG. 6*c*). Because they are not readily detached, they are, like the microconidia of dermatophytes, aleurospores in Vuillemin's sense (15). They may give rise to numerous microconidia which become suspended in the drop exuded by the macroconidium.

We may classify the spores of *Histoplasma* as follows. A *primary* conidium is one that grows upon a hypha (FIG. 2*a* and *c*). A *secondary* conidium is one that grows upon a conidium (FIG. 2*b* and *d*). The diagrams in figure 2 are of microconidia, macroconidia, primary conidia and secondary conidia. The conidial tu-

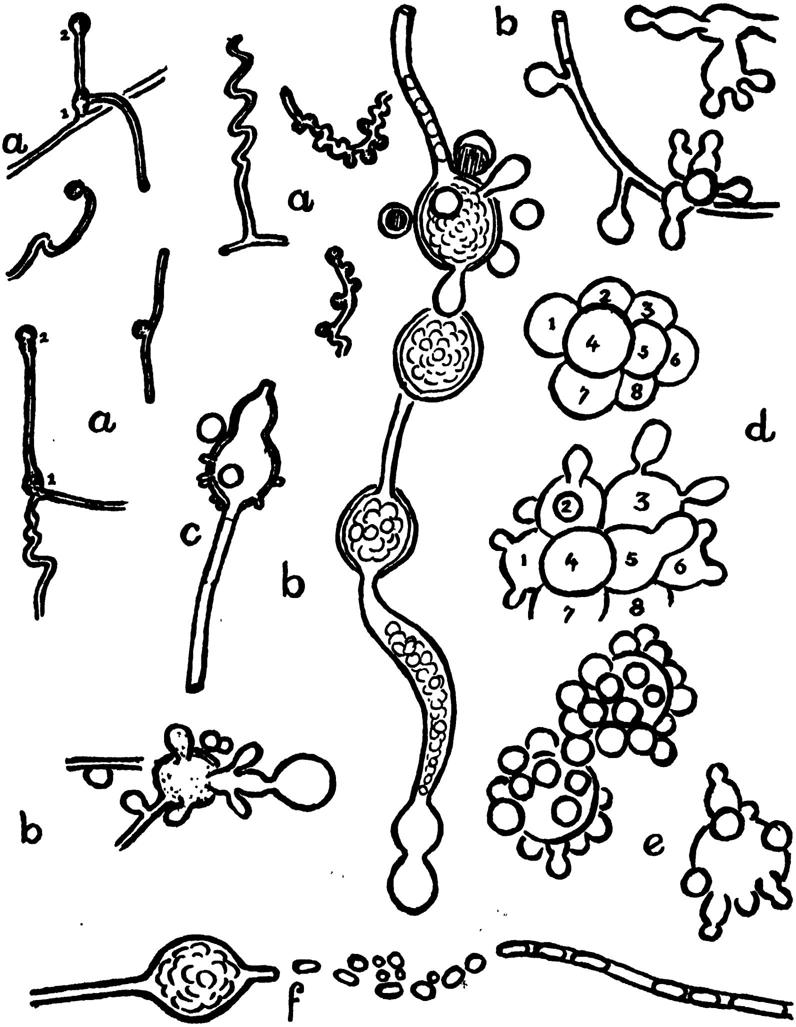

FIG. 3. The spores of *B. brasiliensis: a,* microconidia (shaded), *b–f,* macroconidia; *a* and *e* at 27° C; the rest at 37° C; *d,* growth during an interval of 24 hours. Further description in text. (× 1000.)

berculations (FIG. 2*d*), according to this scheme, are secondary conidia.

Histoplasma grows as a yeast within the host, and also, under certain conditions (**13**), *in vitro*. Conant (**4**) describes the yeast colonies as small, white, moist, and pasty, resembling bacterial colonies. The yeast cell he describes as oval, 1.5–2 × 3 μ, reproducing by a single bud at the pointed end, at maturity enlarging and becoming spherical. Although the yeast cells usually bud singly, in this laboratory cells with as many as five buds have been photographed (**8**).

A previous paper (**8**) described the transformation of hyphae and conidia into yeast cells. In this transition, multiple budding is characteristic of the macroconidium. The secondary conidia which surround it enlarge, become spherical, detach themselves from the parent cell and act as yeast cells (FIG. 6*b*).

BLASTOMYCES BRASILIENSIS

At room temperatures, *Blastomyces brasiliensis* is not vigorous; it grows slowly with a sparse, white, aerial mycelium. The center of young cultures may grow into the air as white tufts or bristles. On Sabouraud's medium cultures could often be identified by (1) their fine radial ridges and (2) the peculiar peripheral zone. This zone, instead of being white and fluffy like the rest, is often transparent and convoluted. Microscopical examination shows that the mycelium here is dead and disorganized. Almeida in describing cultures (**1**), mentions a "hard enveloping mass, a product of the hyphae."

Unlike *Histoplasma* with its tuberculate spores, *B. brasiliensis* on any of the media used, lacks conspicuous distinguishing morphology. The details of the mycelium are obscure. On agar the hyphae are narrow (less than 1 μ in diam.), delicate, indistinct, and tightly interwoven. Frequently lengths of cell wall become dissolved. The globular cell-contents may float in the medium and are then difficult to distinguish from spores (FIG. 3*f*) or they remain as amorphous patches on the surface of the hyphae as described in *Neurospora* (**10**). The most conspicuous hyphae are certain branches that are wider and with denser contents than the rest, sometimes spiral, often hooked, and tending to fuse in pairs.

In general the mycelium suggests abnormal growth due to an unsuitable medium. A medium which furnished a more normal-appearing growth, although without spores, was synthetic asparagin broth with cotton-wool as a source of unsaturated fatty acid (11). The mycelium in this medium was submerged, coarse (with hyphae 2 or 3 μ in diam. and with dense contents), and lacking tangles or hyphal fusions.

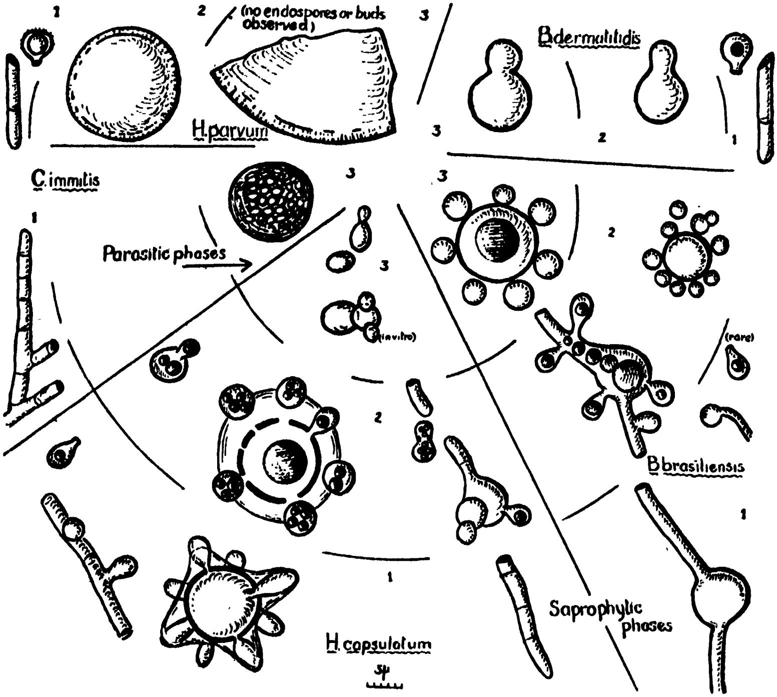

Fig. 4. Five systemic fungi: 1, at 27° C; 2, at 37° C; 3, in host. Reading anti-clockwise, the fungi are: *Haplosporangium parvum, Coccidioides immitis, Histoplasma capsulatum, Blastomyces brasiliensis* and *B. dermatitidis*. Only *C. immitis* has endospores.

The mycelium of *B. brasiliensis* has one feature that serves to distinguish it. It tends to grow in a three-dimensional network (fig. 5*a*). Terminal swellings may produce radially several narrow hyphae which at their tips produce similar swellings (fig. 3*a*, 1 and 2). The resulting mycelium resembles that of the parasite

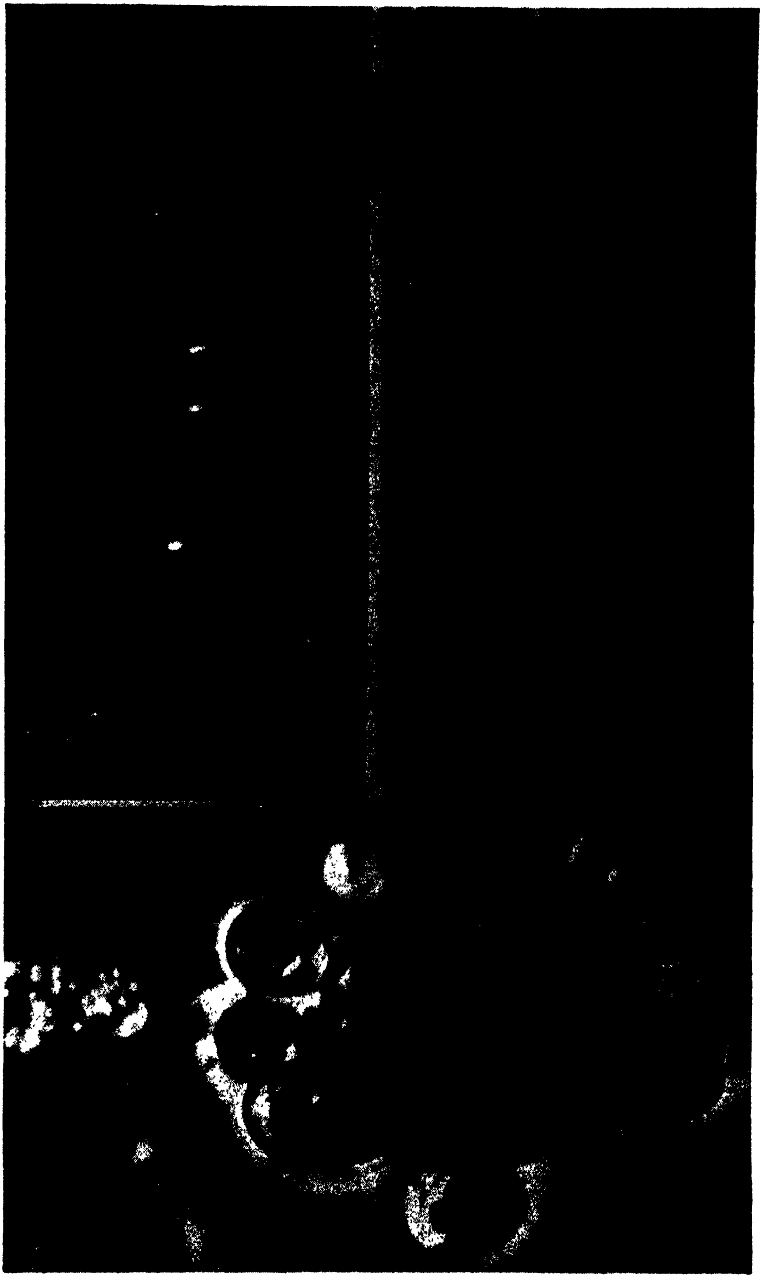

FIG. 5. *B. brasiliensis:* *a* and *b,* on rice at room temperature; *c,* on agar at 37° C; *a,* hyphal reticulum; *b,* microconidia; *c,* macroconidium. (× 1500.)

Syncephalis nodosa van Tieghem which envelops species of *Pilobolus* with a similar network and which is illustrated by Buller (2).

The conidia of *B. brasiliensis* have been described by Conant and Howell (5) as lateral, sessile, round to pyriform, 3–25 μ in diameter.

The writer will term the smaller spores, 1–4 μ in diameter, *microconidia.* They are terminal or intercalary. When terminal, a hypha may grow out from the spore near its point of attachment so that the spore is thrust aside and appears lateral (FIG. 3*a* center). Microconidia are scarce on agar, more numerous on rice. On rice they may be formed in clusters on spiral hyphae, so densely that their arrangement is difficult to make out (FIG. 5*b*). They are spherical and smooth, very seldom pyriform.

Cultures also produce larger terminal or intercalary cells, up to 25 μ in diameter (FIGS. 3*b*; 5*c*). Similar cells are formed in animal tissue. They have been described in the literature as *multiple-budding structures, yeast-forms, arthrospores,* or *chlamydospores.* The writer will term them *macroconidia.* They occur sometimes at room temperature, more readily at 37° C, and are distinguished by their ability to produce multiple buds, although by no means all macroconidia do so.

Compared to the macroconidia of *H. capsulatum,* those of *B. brasiliensis* are about twice the size, more variable in size and shape, and smoother contoured. As Almeida has observed (1), this smooth contour may be broken by small indentations.

At 37° C, *B. brasiliensis* grows as a multiple-budding yeast. Some strains, *e.g.* the English strain 360, are readily converted to the free-yeast form (FIG. 6*e* and *f*). In other strains, *e.g.* Almeida's *B. cerebreformis* now placed with *B. brasiliensis,* the budding cells are not readily detached, but tend to stay together. They grow as a pseudomycelium (FIG. 6*a*) or a tumor-like mass (FIG. 3*b*, upper right, and *d*). Such strains, growing at 37° C as microcultures, were observed successively under the microscope. At first a hypha possessed intercalary macroconidia at intervals along its length (FIG. 3*b*, center). Then the intervening parts swelled into spores, forming a bead-like chain (FIG. 6*a*). The members of the chain then grew by budding (FIG. 5*c*).

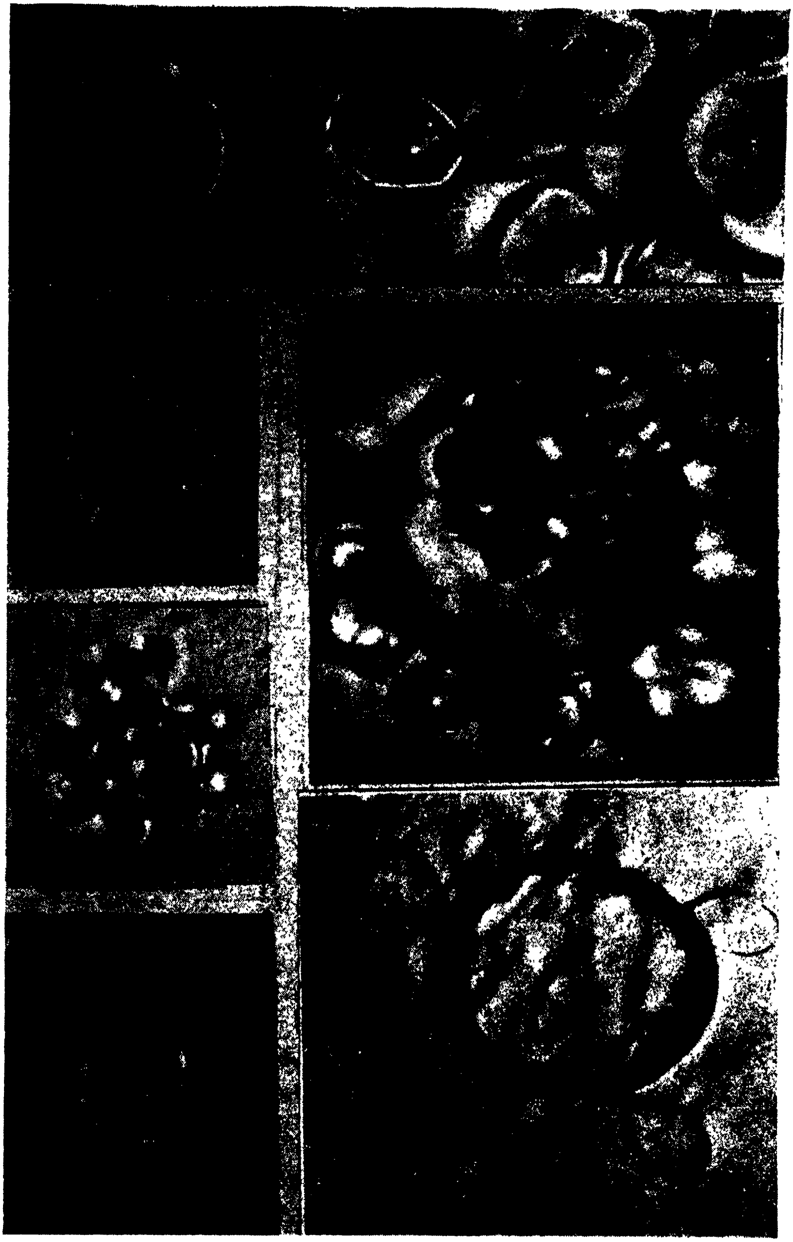

FIG. 6. *B. brasiliensis* and *H. capsulatum* macroconidia: *b* and *c*, *H. capsulatum*, the others *B. brasiliensis*. They are all producing secondary spores. The three spores of *H. capsulatum* in *c* that are slightly out of focus are in reality attached to the macroconidium. (× 1500.)

The multiple buds may be extremely small, even bacilliform (FIG. 3*c*). Sometimes they are difficult to see; for instance only careful examination of the third cell in figure 6*a* will reveal their presence.

DISCUSSION

It is well known that *Coccidioides immitis* reproduces within the host by the formation of *internal* spores, which are set free by the rupture of the spore wall. The ability to produce *external* buds separates the majority of systemic fungi from *C. immitis.* This difference between the species is shown (FIG. 4).

Of the fungi that reproduce by external budding, only *Blastomyces brasiliensis* is differentially diagnosed by its multiple buds in the animal tissue (FIG. 4). However, in the yeast phase of several other species, multiple budding has been observed.[2] Salvin found it occurring under certain cultural conditions in *Sporotrichum Schenckii* (12). De Monbreun (7) has photographed it in cultures from monkeys artificially inoculated with *Blastomyces dermatitidis.* The writer also has observed it in this species. In *Haplosporangium parvum,* figure 2*r* of a recent paper (8) suggests multiple budding.

The tuberculations in the macroconidia of *Histoplasma capsulatum* are interpreted as being external spores. These spores under appropriate conditions act as yeast cells. Multiple budding has, therefore, been demonstrated for an additional species.

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[2] In *Saccharomyces cerevisiae,* Barton (Journ. Gen. Microbiol. **4**: 84. 1950) by observing the bud-scars, has counted as many as 28 times of budding in a single cell.

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NOTES AND BRIEF ARTICLES

CHEMICAL ACTIVITIES OF FUNGI, by Jackson W. Foster. 648 pp. Academic Press, New York, 1949. $9.50.

There are two ways in which a book that covers a rather broad field of biology can be written. In one the author hurriedly assembles a hash of abstracts thickened with a few non-committal words of his own. The other way is for the author to consider carefully the observations reported in the literature of the subject using as a guide the hypothesis he thinks is most successful in unifying the material. This latter way, the one chosen by the author of this book, is the difficult one because knowledge of the subject is required; and much time and effort must be expended to produce the book.

An idea of the scope of the book can be obtained from the titles of the 19 chapters; Introduction, History, Perspective; The Methodology of Mold Metabolism; Chemical Nature of the Mold Mycelium; General Considerations of Mold Metabolism; Natural Variation; Mutations, Physiological Genetics, and Biochemical Syntheses; Trace Element Nutrition of Fungi; Lactic Acid Formation by Fungi; Alcoholic Fermentation by Molds; Oxalic Acid Metabolism; Fumaric and Other C_4-Dicarboxylic Acids; Citric Acid; Itaconic Acid; Kojic Acid; Gluconic and Other Sugar Acids; Carbohydrates Produced by Fungi; Nitrogen Metabolism of Fungi; Other Transformations in Fungi; and Microbiological Aspects of Penicillin.

The first seven chapters (46% of the pages) are devoted mainly to the physiology of the fungi considered in this book. The large amount of space given to the elements of physiology presumably reflects the author's opinion (shared by others) of the low state of current writing about fungous physiology.

In eight chapters (30% of the pages) are discussed acids, alcohols, and polysaccharides formed from carbohydrates. Products, and the possible mechanisms by which they are formed, are the main subjects of these chapters. The fungi are mostly a few species of Rhizopus, Aspergillus, and Penicillium. The activities of yeasts, actinomycetes, and basidiomycetes are not considered.

Until we come to the chapter on penicillin, the contributions of American scientists to chemical activities of fungi are minor when compared with those of the Europeans and Japanese. Penicillin is the only antibiotic substance mentioned, as is proper, since it is the only one of importance formed by fungi. The commercial method for isolating penicillin as outlined in this book is of historical interest only, since the efficient plants use a much simpler process.

On nearly every page there is presented an unsolved problem, sometimes only by implication, so that this book can be used as a source for enough problems to occupy the interest of a generation of graduate students. Amazingly little is known about the details of many of the transformations discussed.

The index, as far as it goes, is excellent and is the kind all worthwhile books should have. Thirteen species of fungi are listed in the subject index. I hope that an organism index as good as the subject index is inserted when the book is reprinted. An organism index would make the work much more useful than it is to the beginning student for whom it was written. Names of fungi mis-spelled in the text should be corrected at least in the index.

Certain aspects of the book must be criticized. The title is more inclusive than the book. Skillful editing would have removed the verbiage and reduced the size of the book or released the space for other material. On many pages indentations apparently were inserted to break up the monotony of a solid page of type; they do not indicate the unity of thought that should constitute a paragraph. The shortcomings of the book are of minor importance. Nearly everyone who works with fungi will find something useful in it and will need to keep a copy handy.—F. W. KAVANAGH, NEW YORK BOTANICAL GARDEN.

MYCOLOGIA VOLUMES WANTED

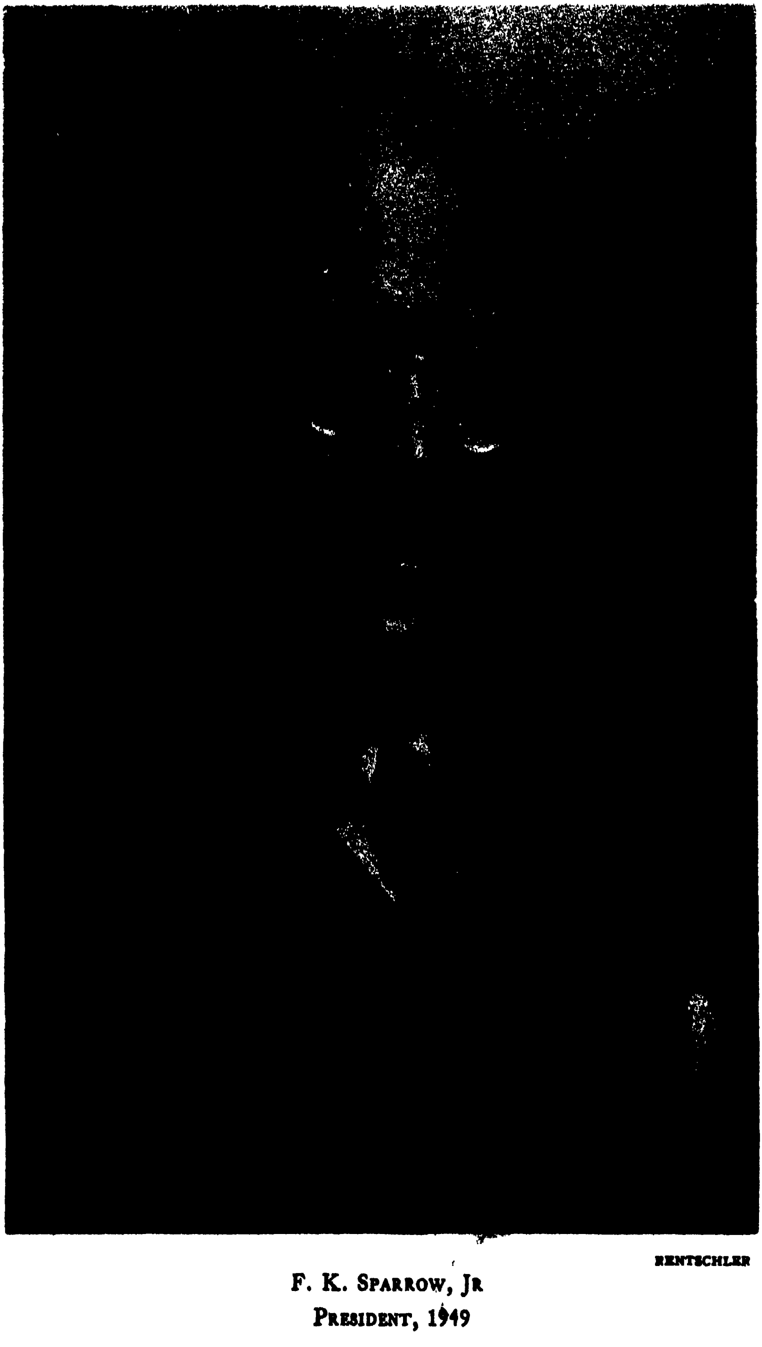
Dr. Juan C. Lindquist, Curator of the Instituto de Botanica Spegazzini, La Plata, Argentina, writes that a fire has destroyed a portion of the Institute's library, although the collections themselves fortunately were not injured. Among other items destroyed were vol. 1, nos. 5 and 6, 1909; vols. 25 and 26, 1933–34; vol. 28, nos. 4 and 6, 1936; vol. 38, no. 1, 1946 and vol. 39, nos. 2 and 3, 1947. Funds are not available at present for purchases of books or periodicals and Dr. Lindquist hopes that anyone having duplicates or unneeded copies of the above volumes will be good enough to send them to the Institute.

MICROCARDS

Microcard copies of Fries's *Systema mycologicum* and *Elenchus Fungorum* have been prepared by the Microcard Foundation, Middletown, Connecticut. The reproduction is sharp and the text can be read with either a dissecting binocular or a hand-lens; Volume I occupies ten 3-by-5 cards. The four volumes are sold for $8.40; Volume I alone is $2.20.—A. H. SMITH.

CORRECTION

In "The Genus Gibellula on Spiders in North America," by E. B. Mains, *pleiopus* should read *leiopus* as used in the following names *Torrubiella arachnophila* var. *leiopus* (pp. 312, 314, 318 and 321), *Gibellula leiopus* (pp. 318 and 321), and *Gibellula arachnophila* f. *leiopus* (p. 318).



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F. K. Sparrow, Jr

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THE EXPANDING HORIZONS OF MYCOLOGY [1, 2]

F. K. SPARROW

In the history of any science certain periods of intense and productive work are easily discernible. Sometimes these are the result of the enthusiasm of an unusually successful teacher or keen research man. Sometimes they come about because of the discovery of organisms ideally suited for certain types of experimentation. Sometimes new tools for the investigation of special problems are discovered. Sometimes because of pressing national necessity, fields of investigation quickly open up that were undreamed of for the immediate future. Sometimes all of these elements combine to produce a relatively rapid reorientation and fresh approach to the science.

The field of mycology, for example, has been given a new impetus and drive in the United States in recent years by some or all of these elements. It would be informative and no doubt interesting to assemble data from our journals of the past 40 years on the changing emphasis in fields of investigations on fungi. It seems sufficient here, however, to put these in general terms.

For many years research in mycology has been primarily morphological and taxonomic, and it has been fruitful work, indeed. To be sure, there has always been an interest in the physiological, genetical, and general "experimental" avenues of approach, but

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[1] Presidential Address, Mycological Society of America, New York, 1949.

[2] Contribution No. 911 from the Botany Department, University of Michigan.

[MYCOLOGIA for September–October (42: 591–682) was issued October 16, 1950]

it has not been great or sustained and has often dealt with specific isolated processes or products. There can be little question but that the excellent fundamental groundwork done in earlier years in the taxonomy and morphology of fungi has been a potent influence in making possible the recent development and expansion almost by leaps and bounds of new fields of investigation.

We are at the moment watching on the sidelines, as it were, a beautiful and what will unquestionably come to be regarded as a classical example of the use of a fungus in unraveling the complicated skein of biochemical syntheses.

The success of the current work on the biochemical-genetics of *Neurospora*, presents to the speculative, general mycologist much food for thought. Here, because of the excellent morphological groundwork laid by Shear, B. O. Dodge and others, a team of investigators, chiefly interested in genetics and biochemistry, and utilizing genetical data, accumulated primarily by Dodge and Lindegren, has underlined a fresh approach to mycological study, the biochemical-genetical attack on fungus metabolism.

To be sure, a great measure of their success is due, as they readily admit, to the special qualities of the fungus itself, and this is a point of more than passing interest. Too long, perhaps, have some of us regarded the activities of our special pets with an unappreciative eye. We must come to a greater realization of the fact that among the fungi are organisms ideally suited for the study of fundamental, universal physiological processes. In *Neurospora,* for example, its simple nutritional requirements and high rate of growth favor biochemical studies. The fact that it is heterothallic and haploid favors a clear interpretation of genetical data since no problems of dominance and recessiveness occur. The linear arrangement of the ascospores in the ascus and their origin facilitates further precise genetical analysis and, since all the ascospores ordinarily are viable and give rise to colonies, no meiotic products are lost. A further favorable feature is the rapidity with which the whole life cycle is completed. The problems of both genetics and biochemistry can be reduced to unusually simple terms.

I need not repeat the fruitful and stimulating results of this particular series of investigations nor emphasize their fundamental biological significance. There is given us here still another line of evidence which points to the universality among living things of basic phenomena, whether they be reduction division or amino acid requirements.

It would be intriguing to speculate on the wider application of the *Neurospora* work. Research in the complex problems of human nutrition and metabolism will beyond question benefit greatly. In our own area of study, biochemical mutants artificially produced might well in the future give us clues as to the origin of parasitism among the fungi.

In the Aquatic Phycomycetes, there exists a field ripe for a new approach. We have up to now been primarily concerned in discovering just what forms are present in nature and in learning something of their morphology, reproductive processes, habits, etc. Very definitely, this work has been in the classical tradition of the older mycology. The prediction I made two decades ago that there must exist an enormous microscopic phycomycetous flora, only a few scattered examples of which we then knew, has been abundantly confirmed. And we have by no means even now exhausted the field of pure discovery, as any attentive reader of the *American Journal of Botany, Mycologia* or the *Transactions of the British Mycological Society* will readily confirm! A modern young Linneaus can, without too much difficulty, with suitable procedures, ferret out whole hitherto unknown or little known groups.

Something more fascinating than the discovery of new species, however, has emerged from these researches on Aquatic Phycomycetes—something which presents to experimental mycology a fertile field for investigation. We read after the routine technical descriptions of new species of chytrids a somewhat bizarre and almost incredible list of substrata: "On chitinous exuviae of aquatic insects," "cellophane," "onion skin," "pollen grains," "green algae," "shrimp skeleton," "snake skin," etc. In other words it is now evident that even in this single group of the Phycomycetes we have an aggregation of microorganisms with vast capacities for the production of diverse enzyme systems and a general metabolic dexterity of a high degree. Further, when we read that a single, well-defined morphological species such as *Catenaria anguillulae* can utilize such a range of substrata as eel worms, liverfluke eggs, algae, snake skin, mosses, ferns and dead angiospermous tissue we appreciate old Haller's well-known and oft-quoted characterization of fungi as ". . . a mutable and treacherous tribe. . . ."

We are only beginning to culture these extraordinary chytrids with any degree of facility. With the advent of rapid, sure methods of obtaining and maintaining them in pure culture, a broad field for physiological investigations will open up which will beyond doubt be one of unusual fruitfulness in both the areas of biochemistry and general nutrition.

It is heartening to see that a successful start has been made on basic physiological problems of Lower Phycomycetes by using modern physiological methods and tools. From what little we now know, their morphological diversity is only equalled by their capacity to derive nutriment from a wide variety of materials.

Research on the genetics of the Aquatic Phycomycetes, however, has been limited by various considerations, not the least of which has been the difficulty of getting some of them into pure culture and maintaining them. Furthermore, before any extensive work can be pursued along genetical lines, methods must be developed for securing ready and abundant germination of the diploid phase by the production of uninucleate zoospores. This has been done with a high degree of success primarily in the Blastocladiales and it is in the genus *Allomyces* of that order that basic genetical work has been initiated and where it will no doubt be most quickly developed. Here, male and female gametangia are readily separated and interspecific hybrids can be produced. Here, also, the resting spore, or its equivalent, can be subjected to outside physical and chemical forces during meiosis, and artificially produced mutants obtained. Furthermore, since the fungus is easily cultured, nutritional studies of a fundamental nature can readily be undertaken. Other features such as its coenocytic nature, rapid rate of growth, simplicity of structure, fast life cycle, etc. make it equal, to my mind, to *Neurospora* for biochemical and biophysical studies.

We have the tools and we have the fungi. We can look forward, now, I believe, to a new era of investigation on Lower Phycomycetes by persons trained in modern physiological methods, using modern tools of attack.

Returning to a more general consideration of mycology, we see on all sides new developments which project the study of fungi into areas little or not at all touched upon in the past. I can mention here only a few of these. There are many others, as for example the work on variability, studies on the production of fats by fungi, the use of fungi in biological assays, etc.

Under the impetus of our recent national emergency, mycology has suddenly expanded into the arena of national defense. The establishment of the Deterioration Laboratories by the Army Quartermaster Corps, organized and guided by the first President of our Society, William H. Weston, proved of great value in solving the immediate problems of materiel deterioration and in meeting new ones. War brought to the fore this somewhat neglected aspect of mycology. There was an almost belated appreciation of the rôle of fungi in the destruction of fabrics, leather, delicate instruments of essential communication, and even lenses. All or nearly all of these deleterious activities of fungi were known beforehand. However, the tremendous scale on which this deterioration of essential war materials took place in a global war, especially as waged in the tropics, pointed up the problem and convinced even the most skeptical of the need for prompt and efficient teamwork.

The work of the Biological Laboratories of the Quartermaster General Laboratories is being continued. This organization embraces laboratories of Microbiology, Biochemistry, Physiology and Biological Testing, all of which are concerned with various aspects of fungus metabolism in relation to the deterioration of military and industrial materials. Many hundreds of cultures of fungi isolated from deteriorating military equipment from all over the world are maintained in active condition both at Philadelphia and at the Farlow Herbarium at Cambridge, Mass., where part of the project is placed. Thousands of others are kept viable but in an inactive state for possible future study. Each fungus in the active collection will eventually be studied in all aspects of its relation to deterioration. Especially significant species which lend themselves to such treatment will be used in biochemical investigations of various processes of deterioration. The results of these continuing studies will be of unquestioned value to the military and to private industry.

The proven value of mycological investigations in solving problems of deterioration in vital naval equipment, has resulted in the formation of a section of Moisture and Fungus Proofing in the Bureau of Ordnance of the Navy Department. In this organization the mycologist has such varied duties as: development of research programs in moisture- and fungus-proofing, both within the Navy and in industrial and research institutions; determination of the extent of damage by fungi and moisture on shipboard; writing specifications for moisture and fungus proof varnishes, etc.; devising tests for materials; consultation with naval establishments and contractor groups; liaison and committee work with groups both within and outside the Navy. The activities of this section demonstrate not only the present importance of mycological work in vital defense but the diversity of duties the modern "practising mycologist" may be called upon to perform.

Let us turn for a moment to another aspect of mycology. It seems hardly necessary today to call special attention to the field of antibiotics. Within a short space of time a new area of fungus study and a giant industry have been developed. This lusty young industry has in many cases been both guided and developed by mycologists who were specialists in fields far removed from their present one. They left their former research interests and with admirable courage turned their energies towards developing and expanding the commercial aspects as well as the purely scientific ones of a new field of mycology, a field of our science of vital importance to every human being. It is plain to see that as a result mainly of their efforts, fungi have definitely entered big business, and they seem there to stay.

As we find out more and more about the workings of these powerful therapeutic agents, we wonder if there may not be in nature many other, undiscovered, strains not only of *Penicillium*, etc., but of other fungi, which might yield products to fulfill diverse clinical needs. Perhaps we have just scratched the surface of this phase of mold metabolism and perhaps there await us, either in nature or in experimentally produced strains, organisms capable of yielding a vast array of substances of both medical and general commercial importance.

Along with this resurgence in the commercial use of molds there has run a parallel expansion of what has been called "fermentation engineering." The growing of large quantities of fungi under sterile, controlled conditions and the refining of their products have brought forth new problems in plant construction, new types of apparatus, machinery, etc. This, too, is a part of the expanding mycological picture, for these engineers are dealing with problems of fungus growth and metabolism on a large scale.

One cannot omit mention at this point of the part played by the Northern Regional Research Laboratory in the development of improved methods of penicillin production. The wartime collaborative efforts of this organization with other centers of research on this problem not only resulted in notable increases in the yield of this valuable drug by way of high-yielding strains, etc., but also materially assisted in putting penicillin production on a sound commercial basis. Wartime governmental agencies considered the penicillin program second only in importance to the perfection of the atom bomb. This same N.R.R.L. group also gave effective service during the war to the studies on deterioration being carried on by the Quartermaster Corps. The wisdom of seeing to it that the Regional Research Laboratories were staffed with able mycologists and microbiologists has been of immeasurable service to the country. At present, the Culture Collection of the Fermentation Division of the Northern Regional Research Laboratory carries over 5000 species and strains of filamentous fungi, yeasts, bacteria and actinomycetes. These are maintained for a dual purpose: to supply microorganisms for their own investigations on fermentations and to supply research laboratories both here and abroad with cultures of significance in fermentations and other fields of microbiology. One of the most important aspects of the collection is the maintenance of multiple strains of particular species which are of known or potential value in important fermentation processes.

We might turn to yet another aspect of our science—Medical Mycology. This area has been gradually assuming greater and greater importance among active practitioners and clinicians and to some degree in medical education. Possibly this has come about because a few well-qualified mycologists and physicians have had a continuing interest in the field. Working closely with those familiar with the clinical aspects of fungus diseases in man they have accomplished a great deal. We can look forward, I believe, to an increasing demand from medical centers for persons trained in basic mycology who will further specialize in medical mycology. How much can be done in an already crowded medical college curriculum towards increasing the time devoted to a study of fungi is problematic.

We might mention at this point the establishment by the government, under the National Institutes of Health, of an organization devoted primarily to the study of large fundamental problems of mycoses in man. This group undertakes field studies on diseases suspected to be of fungus origin. It also carries on extensive surveys of pathogens peculiar to certain areas, with special emphasis on such problems as possible wild hosts. Since most of the fungi causing systemic and fatal mycoses in man are not transmitted to persons in contact with the patient, the epidemiology and the source of the infection become matters of greatest importance. The positive demonstration by these workers of the occurrence of coccidioidomycosis and histoplasmosis in wild rodents goes far towards explaining the peculiar geographic distribution of some diseases. Not only does this laboratory provide a diagnostic and identification service as a practical aid to physicians, hospitals and state health laboratories, but it also carries on work on the improvement in diagnostic methods and the dissemination of information about them. It also pursues studies on skin-testing as a diagnostic method and at present is working on serologic methods of diagnosis and studies of the nutritional requirements and responses of fungi with special reference to serologic methods. Studies of fungicides have also been undertaken. An expansion of this important aspect of mycology, touching as it does on the public health and welfare, seems eminently desirable and would serve the country well.

Other agencies which have contributed to this phase of mycology could be mentioned, for example, certain pharmaceutical houses, but this seems sufficient to illustrate that here, as elsewhere, mycology is on the march and is penetrating more and more extensively and effectively into what were once regarded as peripheral areas of the science.

To my mind, our organization stands at a critical point in its history in relation to these newer developments in mycology. We should give full recognition to the fact that there has arisen what might be termed a "new mycology," which merits our fullest possible cooperation. Unquestionably, morphological and taxonomic work will always be a basic and integral part of our science. This new mycology, however, is a logical outgrowth not only of the experimental trend of biological science in general, but of the times through which we are passing.

What can we do? Few of us, indeed, can pick up the long-faded strands of our early training in chemistry and become biochemists of fungi. More of us, perhaps, can put our hand to other, experimental, aspects of the groups with which we are familiar and make some use of the modern tools of experimentation which have been made available to us. All of us who are teachers can assist in the development of this phase of mycology by acquainting our students with the many opportunities for research in the experimental aspects of the subject and by seeing to it that they are properly prepared in the basic sciences to pursue such researches on fungi.

It seems to me that at this time certain features of the Society should be discussed and decisions made. We ought to have within the framework of the Mycological Society provision for those interested in these new areas of mycology. For example, there might well be a section concerned with research and problems in "mycological" or "microbiological engineering." This expanding field lies, to be sure, on the borderline of our science, yet it *is concerned with fungi. There could be formed with profit to all* a section of the Society dealing with problems arising from the industrial uses of fungi. Those interested in the physiology of fungi are most certainly people who should be members of the Society. Much can be gained by mutual contact, and the views of all will be broadened. Thinking along these same lines, we might well include the field of deterioration and preservation. Here is another group dealing with the applied aspects of mycology. To be sure, many of their problems are problems of chemistry, manufacture, etc., but they do center around fungi. Amateur groups should be encouraged to affiliate with us. To form independent societies concerned with individual facets of mycology results, it seems to me, in a loss to all. The new society centers its interests around a small field and can easily lose contact with the broader aspects of the science. The parent group, in turn, loses the catholicity of interests which an organization of national stature should possess.

It might be pertinent here to emphasize that the Mycological Society of America is not solely a society for the study of fungus taxonomy. That it has seemed like the latter to some, can be traced in part to the fact that the founders of the Society were in large part interested in taxonomy and this was naturally reflected in the activities and publications of the Society.

Let me say, in conclusion, that the Mycological Society, I feel sure, is interested as it always has been, in *all* phases of fungi, and that as the horizons of mycology continue to expand, the Society will keep pace with them.

THE RELATION OF NUTRITION TO THE GROWTH AND MORPHOLOGY OF TRICHOPHYTON FAVIFORME

LUCILLE K. GEORG *

(WITH 6 FIGURES)

The large-spored ectothrix trichophytons of animal origin were described by Sabouraud in 1893 (1) as *Trichophyton faviforme* or "trichophytons of faviform culture" because of the resemblance of their colonies to those of *Trichophyton Schoenleini,* the causative agent of the disease favus. These fungi were extremely slow to develop on the sugar-peptone media of Sabouraud and, even after 3 or 4 weeks of growth, produced button-like colonies only 10 to 15 mm. in diameter. The colonies were usually raised and often acuminate (occasionally flat), with an irregularly folded surface. They were tough and leathery in texture. The surface was glabrous and at times moist, with a small amount of white powder or velvety surface growth developing on older colonies. Microscopic examination revealed a highly irregular, twisted, degenerate type of mycelium with many chlamydospores. Microconidia and macroconidia, the characteristic spores of the genus *Trichophyton,* were not observed. Although their cultural appearance resembled that of *T. Schoenleini,* Sabouraud separated these fungi from the latter because of their common occurrence on cattle and horses, their ectothrix appearance clinically, and their association with circinate or suppurative lesions typical of ringworm rather than of favus.

E. Bodin (2), who studied the culture described by Sabouraud as *Trichophyton faviforme,* isolated several similar organisms from lesions on horses, a donkey, and a calf. In addition, Bodin described a strain which he at first called "Trichophyton faviforme

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du veau" but later named *T. verrucosum* (1902). His description was as follows: "On glucose agar the culture forms a little cake in a month's time. Part is submerged in the agar, and the surface is irregular, verrucose, and grey."

In 1908, Sabouraud (3) described two species in this group. One which he called *Trichophyton ochraceum* corresponded closely to the *T. verrucosum* of Bodin except that on maltose agar it formed a yellow ochre pigment. The second species, *T. album,* was difficult to distinguish from *T. Schoenleini.* It formed a glabrous, acuminate colony with regularly folded sloping sides. Some colonies were umbilicate and resembled a miniature volcano. Sabouraud stated that it was extremely slow-growing and poorly developed in comparison with cultures of *T. Schoenleini.* He found that primary cultures had to be placed in an incubator at 37° C. to obtain best development. After continued cultivation, the organism occasionally developed a fine white powder, or an irregularly distributed short white down over the surface of the colony. In 1910, Sabouraud (3) described a fourth species, *T. discoides,* which he stated was similar to *T. album* except that, in culture, it formed an almost perfect disc with a flat surface and often a central knob. Some colonies had a slight brownish color and a moist appearance while others developed a fine, short white velvety growth over the entire surface.

Many workers have studied the organisms of the so-called "faviform group" in an attempt to obtain spores which would identify them more clearly as members of the genus *Trichophyton.* The introduction of the "natural media of polysaccharide base" by Langeron and Milochevitch (4) has aided this study greatly. On these media, which consisted of whole grains of wheat, barley, corn, and oats, the fungi developed quite rapidly, producing an appearance entirely different from that of the glabrous colonies obtained on the sugar-peptone media of Sabouraud. A white powdery or even fluffy growth spread over the surface of the grains and microscopic examination revealed microconidia, characteristically borne singly along the hyphae (en thyrse) and terminally in clusters (en grappe). Rudimentary spirals and one abortive macroconidium typical of the *Trichophyton* genus also were demonstrated.

In 1934, Lebasque (5) carefully reviewed the "faviform group" and was able by the use of the polysaccharide media to complete morphological studies of the known species as well as of three new species. Lebasque accepted the following as valid members of the genus *Trichophyton: T. verrucosum* (Bodin, 1902), flat, with verrucose surface and a grey color, *T. ochraceum* (Sabouraud, 1908), flat and verrucose, but with an ochraceous pigment; *T. album* (Sabouraud, 1908), heaped and folded, with a waxy appearance; *T. discoides* (Sabouraud, 1910), disc shaped with a small central knob; and *T. equinum* (Gedoelst, 1902), and *T. caballinum* (Neveu-Lemaire, 1921), two strains isolated regularly from horses, which showed a white, downy to fluffy surface but were otherwise similar to other strains in this group in their lack of ability to produce spores on the sugar-peptone media. His three new species were *T. bullosum, T. villosum,* and *T. papillosum.*

Since many of these species had been designated solely on the basis of colony size and shape, degree of downiness, and pigment production, characteristics which are highly variable among dermatophytes, it is probable that several are actually synonymous. It seemed important to study a series of recently isolated strains in this group as well as cultures from stock collections in order to learn the range of variation which could be expected in a single strain.

Furthermore, since it has been shown that under certain conditions these fungi can grow rapidly and produce spores, it was hoped that more comprehensive studies on their nutritional requirements would establish their characteristic types of growth and spore production and thus furnish a firmer basis for their identification.

EXPERIMENTAL

Part I. Cultural Studies of Recently Isolated Strains of T. faviforme

The strains studied were isolated from cases of ringworm which had appeared among a group of farmers and their families in central Pennsylvania. In most cases large-spored, ectothrix involvement of the hairs had been observed, and the lesions, which occurred most frequently on the face, neck and scalp, were of a deep suppurative type. In all instances there was a history of recent contact with cattle, and in several cases similar cultures were isolated from ringworm lesions on these animals. The clinical course and treatment of a series of 23 cases have been described in a previous publication (6). It was pointed out that it was occasionally difficult to isolate the fungi from these lesions using Sabouraud's dextrose agar and incubating at room temperature. However, by the use of a heart infusion-tryptose agar (Blood Agar Base, Difco) and incubation at 37° C. the chances for successful isolation were much improved. Although the fungi developed rapidly on this medium, growth was enhanced further when 0.1 mg. thiamine was added to 100 ml. of the melted partially cooled agar. On heart infusion-tryptose agar with added thiamine, the colonies were 30 to 40 mm. in diameter at the end of two weeks. They were often heaped and folded and covered with considerable surface growth which appeared as a fine powder or an erect white down. Microscopic examination of such colonies revealed a regular, flexuous mycelium with only occasional chlamydospores. The aerial growth contained large numbers of microconidia, and in several strains characteristic macroconidia were found as well.

A. Growth on Sabouraud's dextrose agar

On Sabouraud's dextrose agar, the fungi grew slowly and produced small, glabrous colonies resembling the species described by Sabouraud as *T. ochraceum, T. album* and *T. discoides* (FIG. 1, a, b, c). Microscopically only irregular mycelium and chlamydospores were observed (FIG. 2a). With frequent subculturing the strains could be maintained on Sabouraud's dextrose agar, but the colonial form was not stable. It was decided to study a series of single spore isolates in order to observe the possible range of variation within each strain.

Eight strains showing various colonial forms were selected for study. Spore suspensions were obtained from growth on the heart infusion-tryptose-thiamine medium described above, and six microconidia were selected from each strain according to the method described by Georg (7). The monospore cultures were studied over a period of several months. Some of the strains were quite stable and retained their original colonial form even after several

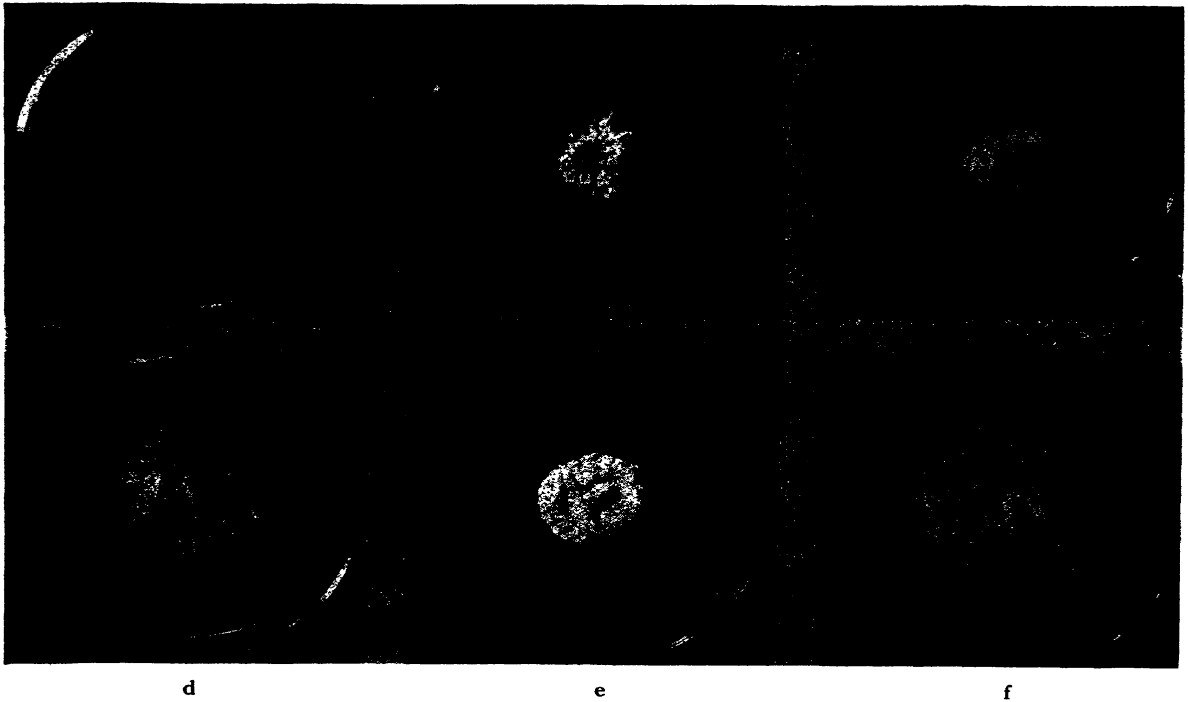

FIG. 1. *a, b, c, T. faviforme* (varieties *ochraceum, album* and *discoides*) on Sabouraud's dextrose agar; *d, e, f,* Same cultures on trypticase-dextrose-thiamine agar.

transplants on Sabouraud's dextrose agar, but the large majority were variable, producing a wide range of colony forms. Figure 3 indicates some of the variants obtained from a single spore strain. Not only were there differences in shape and topography of the colonies, but also in the presence or absence of aerial growth and of pigment.

The possibility of variation in these organisms was first suggested by Cazalbou in 1913 (8) when he described *Trichophyton singulare,* a new species which had two cultural states, one glabrous and cerebriform, the other downy, flat, and disc shaped. The two forms were found to be reversible. In 1938, Gammel and Work (9) described a case of ringworm contracted from cattle and caused by a fungus which they called *Trichophyton album* var. *singulare.* After several transplants on a liver infusion agar, this organism produced two distinct types of growth, a cerebriform and a discoid.

These findings as well as our data obtained with single spore cultures seem to indicate that *Trichophyton album, T. discoides,* and *T. ochraceum* are not specifically distinct. It is suggested that the name *Trichophyton faviforme* be retained for the variants in this group. *T. faviforme* was the first designation employed by Sabouraud (1) and also by Bodin (2). It has had considerable usage, and is more generally descriptive of these organisms than names which represent highly variable colonial characteristics. The colony types may be designated as varieties: album, discoides, ochraceum, etc.

B. Growth on whole grains

The eight monospore strains of *Trichophyton faviforme* were planted on moist rice and barley grains. All the strains grew well, covering the grains with a felt-like mat. By the tenth day, most of the cultures had formed an erect aerial mycelium which extended beyond the kernels. A few of the strains produced only a fine, white, powdery growth at first but by the twenty-fifth day some down was visible here also. Microscopic examination showed regular, branching, flexuous hyphae with occasional intercalary and terminal chlamydospores. The aerial growth contained numerous microconidia scattered along the mycelium, as well as in

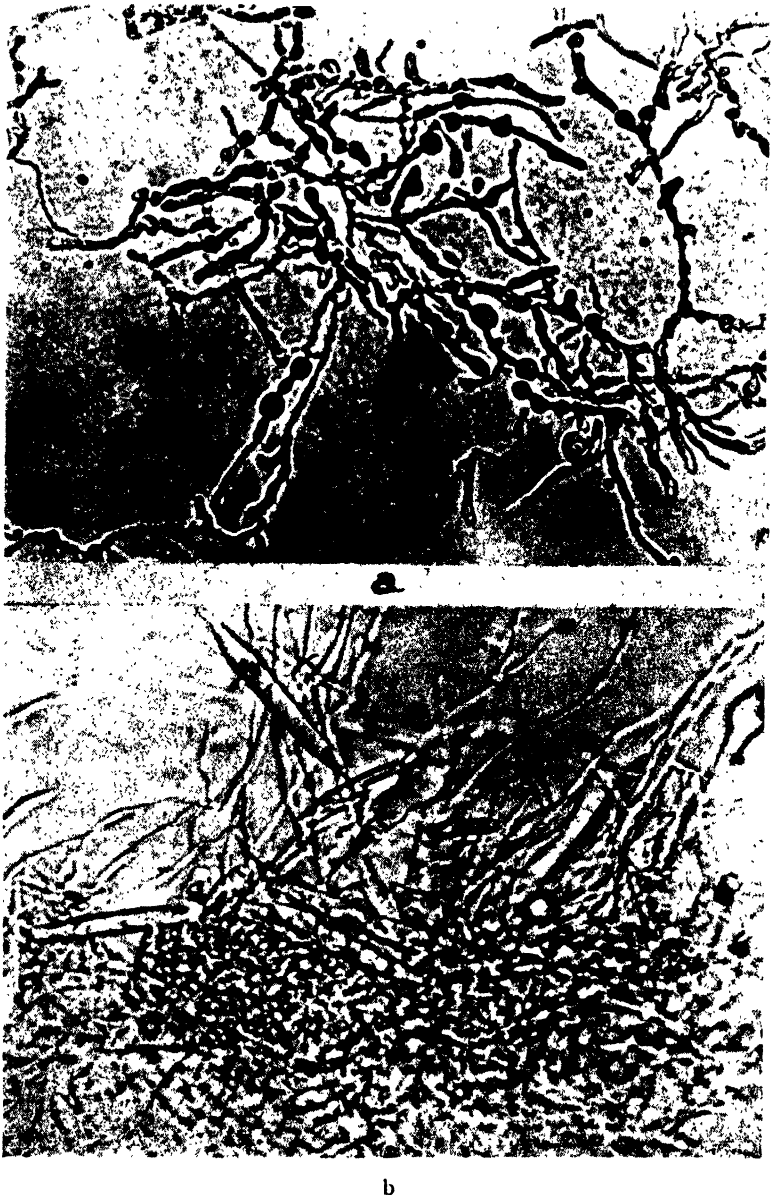

FIG. 2. *a*, Microscopic appearance of *T. faviforme* from growth on Sabouraud's dextrose agar; *b*, The same culture from growth on heart infusion-tryptose-thiamine agar.

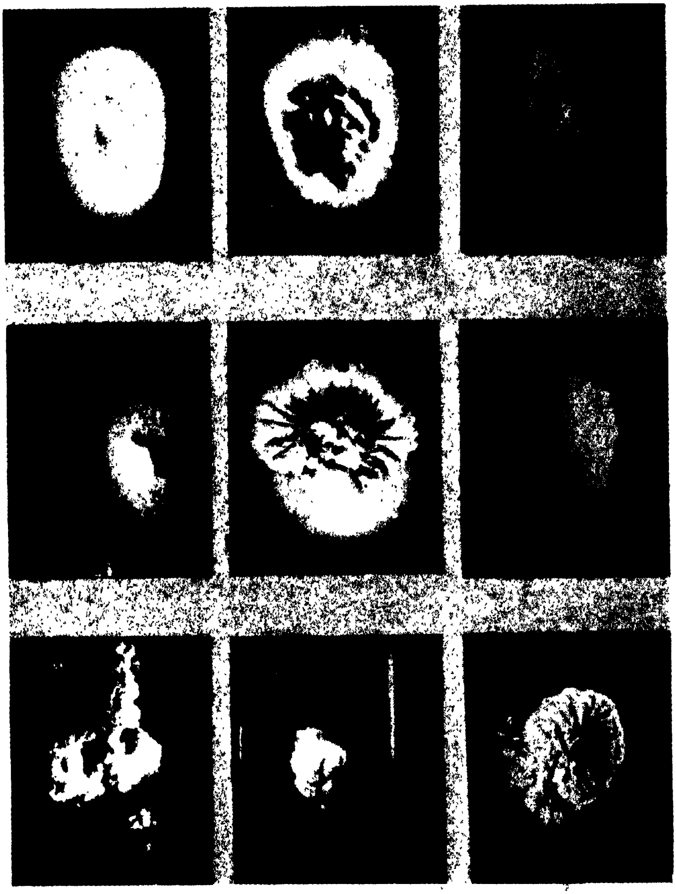

FIG. 3. Variation in colony form in a single spore strain of *T. faviforme*—Sabouraud's dextrose agar.

terminal tree-like clusters (FIG. 4a & b). These spores broke off squarely and easily from the point of their perpendicular attachment and were generally rather slender and elongate with considerable variation, measuring 1.5–2 × 3–4 μ.

Macroconidia were found also in the downy to fluffy aerial mycelium appearing from the tenth to thirteenth day. These were thin walled, 20–50 × 5–8 μ, with 1–7 septa, occurring singly either laterally or terminally. Some were short and bulbous, others had

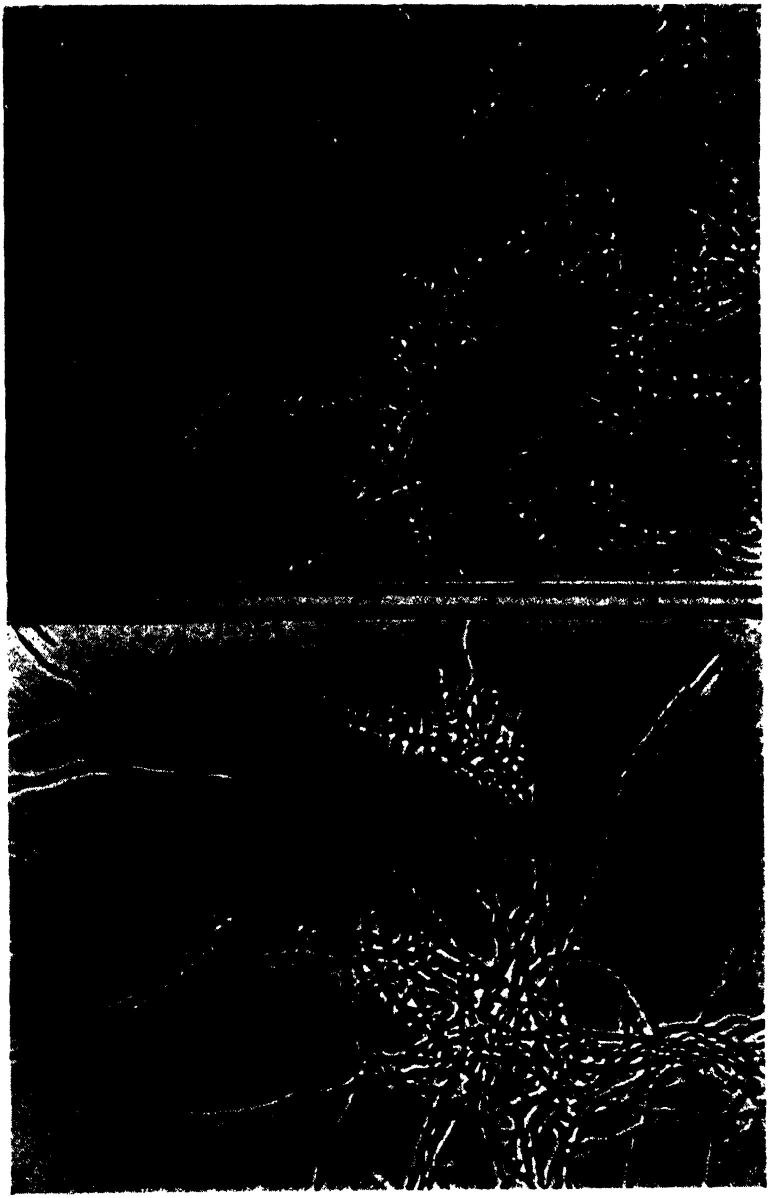

Fig. 4. *a*, Microconidia scattered along the mycelium from growth on rice grains; *b*, Microconidia in terminal clusters from growth on rice grains.

long pointed ends, and still others were irregularly swollen and distorted (FIG. 2b).

C. Growth on enriched media

Growth was stimulated by both yeast extract and liver extract, the amount of aerial mycelium produced depending upon the strain as well as on the quantity of extract available. On Sabouraud's dextrose medium to which had been added 0.1 per cent of either extract, all strains grew rapidly, producing thick, compact colonies with a heavy, white, powdery, or occasionally white, fluffy, surface growth. Microconidia were numerous, and macroconidia appeared in some strains.

The stimulatory effects of Bacto-tryptose, beef heart infusion, and citrated human blood were studied in view of the fact that in the isolation work, blood agar plates had been used and found to be superior to Sabouraud's dextrose or maltose agar (6). It was thought at first that the whole blood in this medium was the stimulating factor. However, no difference was detectable in the amounts of growth on Blood Agar Base with and without the addition of whole blood. Both Bacto-tryptose and beef heart infusion were found to produce a stimulating effect when added to Sabouraud's dextrose agar in amounts comparable to those found in the Blood Agar Base. Trypticase, an enzymatic hydrolysate of casein (Baltimore Biological Co.), was studied also in an effort to find a practical, easily reproducible medium for routine isolation of and spore production by these strains. A one per cent trypticase-dextrose-thiamine agar was found to be satisfactory. On this medium there developed rapidly growing colonies with a white, powdery, surface growth or with a short, erect, white, aerial down bearing numerous microconidia and, in some strains, macroconidia (FIGS. d, e, & f).

The following media are recommended for isolation and production of rapidly growing, sporulating cultures of *T. faviforme:*

1. Heart infusion-tryptose-thiamine agar

Beef heart, infusion from	500 g.
Bacto-tryptose	10 g.
Sodium chloride	5 g.
Bacto-agar	15 g.
Thiamine hydrochloride	10 mg.
Dist. water	1000 ml.

The ingredients are dissolved in the water, the pH adjusted to 6.8, and the medium autoclaved at 121° C. for 10 minutes.

This medium is similar to Difco Blood Agar Base, except for the thiamine. It may be prepared by adding thiamine to the dehydrated product.

2. Trypticase-dextrose-thiamine agar

Trypticase (Baltimore Biol. Co.)	10 g.
Dextrose	40 g.
Thiamine hydrochloride	10 mg.
Agar	20 g.
Dist. water	1000 ml.

The ingredients are dissolved in the water, the pH adjusted to 6.8, and the medium autoclaved at 121° C. for 10 min.

Since both of the above media will support bacterial growth, it is recommended that penicillin, 20 units per ml., and streptomycin, 40 units per ml., be added to the melted, partially cooled agar before pouring into tubes or plates.

For primary isolation, all tubes should be incubated at 37° C. When satisfactory growth is obtained, subcultures may be made to Sabouraud's dextrose agar and the cultures grown at room temperature in order to obtain the characteristic slow-growing, glabrous colonies usually described for this species.

Part II. Studies of the Vitamin Requirements of T. faviforme

The increased growth and spore production of *T. faviforme* on natural media suggested that these substances are rich in growth factors which may be required by this fungus for its optimal development. The stimulating effect of thiamine added to routine media has been discussed above. Recent studies using synthetic, chemically defined media have shown, in fact, that some strains of *T. faviforme* do require certain of the vitamins, and are stimulated by other growth factors present in natural products.

Schopfer and Blumer (10), in their studies of the growth requirements of an organism which they had isolated and classified as *T. album,* have shown that this strain, although able to grow to some extent on a basal medium without the addition of any vitamin, produced a more rapid growth when biotin was added. This was especially true when a nitrogen source such as ammonium citrate was used in place of asparagine. They also demonstrated that, in an unbuffered asparagine medium, thiamine, i-inositol, and pyridoxine clearly furthered development.

Robbins, Mackinnon, and Ma (11) studied a Uruguayan strain of *T. discoides* (Mackinnon No. 688) and demonstrated that it required pyridoxine, i-inositol, and molecular thiamine for growth on a basal medium with asparagine as a nitrogen source. Development of this strain was further stimulated by other factors present in complex products such as peptone, casein hydrolysate, hydrolyzed egg albumin, malt extract, gelatine, and a filtrate from white potatoes.

Burkholder and Moyer (12) have shown that a strain which they designated as *T. faviforme* required only two vitamins, thiamine and inositol. They obtained further stimulation by the addition of liver and peptone to their media.

Mackinnon and Artagaveytia-Allende (13) recently compared the vitamin requirements of seven Uruguayan strains. Of these, four required i-inositol, one thiamine, one i-inositol and thiamine, and the seventh (No. 688), previously studied and reported (11), required i-inositol, thiamine, and pyridoxine. It was shown that the vitamin requirements depended on the individual strain, and were not related to any particular colonial form.

A. Growth on basal agar with vitamin additions

A study was made to determine the vitamin requirements of twenty recently isolated strains of *T. faviforme* (including all three colony forms: varieties album, discoides and ochraceum) as well as of four stock strains: *T. album* (Baudet and Stuhmer), *T. discoides* (Papegaay), *T. ochraceum* (Boedijn), obtained from the Central Bureau for Fungus Cultures, Holland, and *T. discoides* (No. 688), kindly sent from Uruguay by Dr. Juan E. Mackinnon.

A basal agar was made as follows: 2 g. asparagine (recrystallized 3 times from alcohol), 50 g. dextrose, and 0.1 g. $MgSO_4 \cdot 7H_2O$ dissolved in 1000 ml. Sorenson's phosphate mixture at pH 7 (M/15 KH_2PO_4 and M/15 Na_2HPO_4). To this was added 1.5 per cent agar (purified according to the method described by Robbins, 14), and the medium was then autoclaved for 10 min. at

121° C. Seitz-filtered vitamin solutions were prepared at the beginning of each experiment, and equal volumes containing vitamins in varying concentrations and combinations were added to tubes containing 10 ml. of the melted and partially cooled agar. The tubes were then rotated and slanted.

Washed inoculum was obtained from growth in heart infusion broth. It was necessary to wash the mycelium five times before the wash water was free of thiamine. (This was determined by testing the filtrate for ability to support the growth of washed spores of *Phycomyces Blakesleeanus,* which will grow in the presence of minute traces of thiamine.) A small fragment of washed inoculum was placed on the slanted surface of the basal agar with and without the various vitamins tested. All cultures were grown at room temperature for 10 weeks.

The growth studies indicated that: 1. Twenty-one of the strains (twenty recently isolated strains as well as *T. discoides* (Papegaay)) required i-inositol and molecular thiamine; 2. One strain *T. discoides* (No. 688) required pyridoxine in addition to i-inositol and thiamine; and 3. Two strains, *T. album* (Baudet and Stuhmer) and *T. ochraceum* (Boedijn), had no essential vitamin requirements and were not stimulated by additions of vitamins to the basal agar.

For the twenty-one strains which required both inositol and thiamine, inositol when tested alone showed some slight effect. No effect was observed in tubes containing less than 10 μg. inositol per ml. but with quantities between 10 and 100 μg. per ml. the mycelium developed to a radius of 2 to 10 mm. from the point of inoculation. This was a thick, fern-like subsurface growth. Further increments of inositol did not increase the amount of growth. 100 μg. inositol per ml. was therefore considered the maximum effective dosage of this vitamin in an asparagine basal agar. Thiamine showed no effect when tested alone at a concentration of 5 μg. per ml. nor even when increased to 500 μg. per ml. However, in the presence of 100 μg. of inositol per ml., 5 μg. of thiamine permitted rapid development of the cultures. The colonies were heaped and frequently folded, with either considerable white surface powder or velvety aerial mycelium. Further increments of thia-

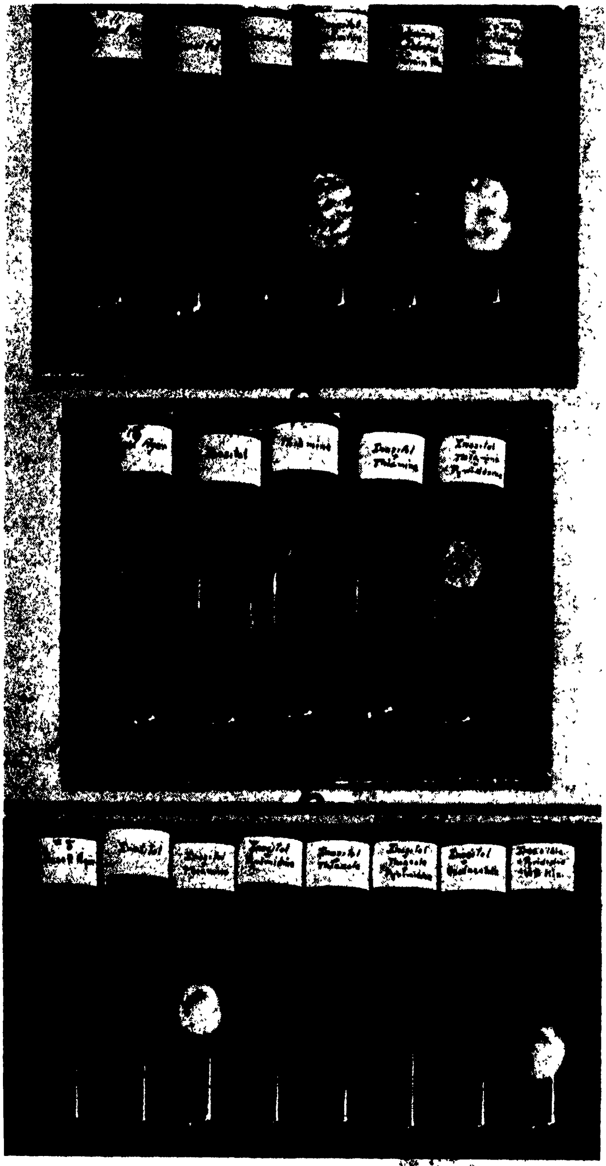

FIG. 5. Strains of *Trichophyton*.

mine did not increase growth. Five μg. of thiamine per ml. was considered the maximum effective dosage of this vitamin in the asparagine basal agar in the presence of 100 μg. of inositol. None of the other vitamins tested (calcium pantothenate 100 μg. per ml., riboflavin 100 μg. per ml., para-aminobenzoic acid 100 μg. per ml., nicotinamide 100 μg. per ml., biotin 0.05 μg. per ml., choline chloride 100 μg. per ml., and folic acid 100 μg. per ml.) showed any effect when tested singly or in all possible combinations, or when added with the maximum effective doses of inositol and thiamine (FIG. 5a).

The finding that *T. discoides* (No. 688) requires pyridoxine in addition to inositol and thiamine is in accord with the report of Robbins, Mackinnon and Ma (11) who also had studied this strain. Inositol showed some slight effect when tested alone in amounts over 10 μg. per ml., but neither thiamine nor pyridoxine showed any effect except in the presence of the other two essential vitamins. In the presence of the maximum effective doses of inositol and thiamine, 5 μg. of pyridoxine per ml. produced rapid growth of this strain. Five μg. per ml. was considered the maximum effective dosage of this vitamin in the asparagine basal agar. None of the other vitamins tested showed any stimulating effect (FIG. 5b).

In contrast to all of the other isolates two strains, *T. album* (Baudet and Stuhmer) and *T. ochraceum* (Boedijn), were found to have no essential vitamin requirements and grew well on the basal agar. No stimulation was observed following the addition of inositol, thiamine, pyridoxine, or any of the other vitamins tested.

For all of these strains, basal agar alone, or basal agar with the maximum effective quantities of required vitamins, was not sufficient to produce growth comparable to that obtained on certain natural media. Bacto-neopeptone, beef heart infusion, Bacto-tryptose, yeast extract and liver extract not only replaced the essential vitamins, where these were required, but in all cases produced a heavier and more rapid growth. This suggests that there are other important nutritional factors present in natural substances which stimulate the growth of this fungus.

Substitution of NH_4Cl, 2 g. per liter, or an acid-hydrolyzed casein,* 1 g. per liter, for the asparagine of the basal agar did not change the vitamin requirements of any of the strains, but growth with NH_4Cl was considerably less than with asparagine or casein hydrolysate.

It was found that neither pyrimidine, as 2-methyl-5-ethoxymethyl-6-aminopyrimidine; or thiazole, as 2-amino-thiazole; or an equimolar combination of these two, could substitute for molecular thiamine for any of the strains studied. It was found also that oxalacetate could not be substituted for molecular thiamine (FIG. 5c).

B. Characteristics of the growth on basal agar with vitamin additions

The twenty-one strains which required inositol and thiamine produced vigorously growing colonies on the asparagine basal agar to which maximum effective amounts of these two vitamins had been added. The colonies were compact and generally covered with a thick, white, velvety to fluffy surface growth. In some instances a yellow pigment developed in this surface growth as the culture aged. The mycelium appeared to be more regular and better developed than the poorly formed, irregular, twisted mycelium observed in the glabrous colonies on Sabouraud's dextrose agar, and the number of chlamydospores was greatly reduced. All these strains developed microconidia, and in some these were very abundant (FIG. 6a). On the whole, they were more numerous than on the rice grains, on which medium these structures have been described in detail above. Macroconidia were produced, usually in small quantities, by all of these strains on the inositol-thiamine-enriched basal agar. They were characteristically irregular in size and shape, and appeared similar to the macroconidia described on rice grains. One strain produced abundant macroconidia which were long and slender with tapering ends (FIG. 6b).

When 2 g. of NH_4Cl per liter was substituted for asparagine in the vitamin-enriched basal agar, the character of the growth and the number of spores produced were not altered appreciably. However, best growth and spore production were obtained when casein hydrolysate was employed as the nitrogen source.

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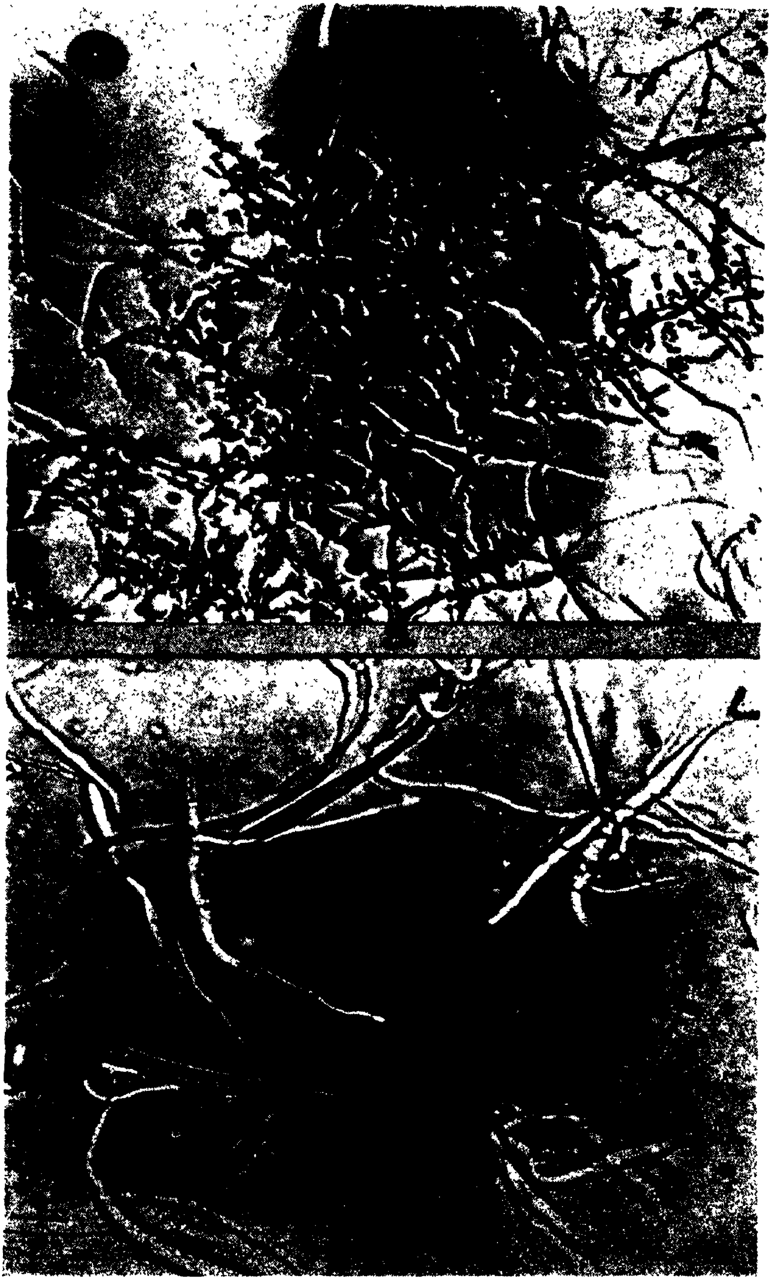
* "Vitamin-free" casein, General Biochemicals, Inc., Chagrin Falls, O.



b

Fig. 6. Strains of *Trichophyton*.

C. *Quantitative studies in asparagine basal broth with vitamin additions*

Several strains were employed to determine the amount of growth which could be produced in a liquid synthetic medium with additions of the required vitamins, alone and in combinations; as compared with that obtainable in a natural medium, Difco Heart Infusion Broth. A basal broth was prepared similar to the asparagine basal medium described above except for the absence of agar. Fifty ml. amounts of broth were placed in 150 ml. Erlenmeyer flasks, and freshly prepared vitamin solutions were added in amounts determined from studies on asparagine basal agar: inositol 100 μg. per ml., thiamine 5 μg. per ml., and pyridoxine 5 μg. per ml. Strains No. 1, No. 5, No. 17, No. 18, and No. 19 were chosen as test organisms. Strains No. 1 and No. 5 were recently isolated cultures both of which had shown requirements for inositol and thiamine. Strain No. 17 (*T. discoides* (Papegaay)) also required inositol and thiamine. Strain No. 18 (No. 688, Mackinnon) required inositol, thiamine, and pyridoxine, and strain No. 19 (*T. album,* Baudet and Stuhmer) had no essential vitamin requirements. The flasks were inoculated with tiny shreds of washed mycelium. The cultures were incubated at room temperature and shaken at intervals to maintain a uniform, submerged type of growth. A series of flasks containing 50 ml. of heart infusion broth with and without added vitamins was treated similarly. After ten weeks, the entire contents of the flasks were filtered through sintered glass cups and the retained mycelium washed and then dried for 2 hours at 110° C. The weight of the mycelium was then determined.

Growth of the vitamin-requiring strains corresponded well with that obtained on the basal agar slants in the previous experiment. The strains which had shown growth with inositol and thiamine showed even more clearly here that these vitamins were required, and that the addition of any of the other vitamins tested had no effect on the amount of growth. The weight of mycelium produced with inositol alone, never more than 2 mg., corresponded to the small amount of subsurface growth which had been observed on the basal slants with this vitamin. For all strains, approximately twice as much growth was produced in the heart infusion broth as in the basal broth with added vitamins. However, even more growth was obtained when the same doses of the required vitamins were added to the heart infusion broth. This indicated that the heart infusion broth did not contain the required vitamins in sufficient amounts to produce maximum growth in this medium. Table 1 gives a summary of the results of these tests.

Titrations were made in the asparagine basal broth to determine the minimum concentrations of the essential vitamins that would stimulate the growth of these several strains of *T. faviforme.*

Asparagine basal broth, prepared as described above, was placed in 10 ml. amounts in test tubes (150 × 19 mm.) and sterilized by autoclaving. Ten ml. of the vitamin to be tested was added aseptically to one of these tubes and diluted serially through 30 tubes, the amount of vitamin being halved by each transfer. The following titrations were carried out for each strain: 1. Titration with inositol alone; 2. Titration of inositol in the presence of thiamine (50 μg. thiamine being added to each tube at the completion of the dilution process); 3. Titration of thiamine in the presence of inositol (1000 μg. of inositol being added to each tube at the completion of the dilution process); and 4. Titration of pyridoxine in the presence of both inositol and thiamine. Control tubes contained asparagine broth, or the basal broth with each vitamin singly and in all combinations in maximum effective amounts. All tubes were brought to equal volume with sterile distilled water. Inoculations were made with fragments of washed mycelium and the cultures were allowed to grow at room temperature for 6 weeks.

Titration 1 indicated that for strains No. 1, No. 5, and No. 18, inositol showed some slight effect alone. The amount of growth was very small and appeared to be the same in all tubes showing growth. The minimal effective concentration ranged from 12.2 to 97.5 μg. per 10 ml. of asparagine broth, depending on the strain. Titration 2 showed, for strains No. 1 and No. 5, that in the presence of adequate quantities of thiamine the amount of growth was greatly increased, and tubes bearing the same dilutions of inositol which alone had produced only a small submerged fluffy ball of mycelium, were now filled with growth. Growth fell off sharply

TABLE 1

COMPARISON OF GROWTH OF *T. faviforme* ON BASAL ASPARAGINE BROTH AND HEART INFUSION BROTH WITH AND WITHOUT VITAMINS

Strain	Thiamine 5 µg./ml.	Pyridoxine 5 µg./ml.	Inositol 100 µg./ml.	Inositol and thiamine	Inositol, thiamine, and pyridoxine	Thiamine-pyridoxine + "vitamin mixture"	Inositol-thiamine-pyridoxine + "vitamin mixture"	Heart infusion broth	Heart infusion broth + inositol thiamine pyridoxine	Basal broth (control)
No. 1 (album variety) Penna.	0	0	less than 1 mg.	14.61 mg.**	14.39 mg.**	0	15.96 mg.**	21.49 mg.**	39.06 mg.**	0
No. 5 (discoides variety) Penna.	0	0	1.59 mg.**	17.51 mg.**	18.34 mg.**	0	18.16 mg.**	45.02 mg.**	91.70 mg.**	0
No. 17 T. discoides (Papegaay) Holland	0	0	1.76 mg.**	29.17 mg.**	28.96 mg.**	0	27.60 mg.**	48.65 mg.**	85.15 mg.**	0
No. 18 T. discoides (Mackinnon) Uruguay	0	0	1.4 mg.**	1.35 mg.**	19.70 mg.**	0	17.17 mg.**	37.15 mg.**	56.85 mg.**	0
No. 19 T. album (Baudet-Stuhmer) Holland	29.18 mg.*	27.62 mg.*	30.0 mg.*	31.19 mg.*	30.45 mg.*	27.15 mg.*	25.20 mg.**	72.42 mg.*	73.83 mg.*	28.23 mg.**

* Average of 4 weighings—dried mycelium from 4 flasks.

** Average of 6 weighings—dried mycelium from 6 flasks.

"Vitamin mixture": (µg. per ml.), pantothenate 100, riboflavin 100, biotin 0.05, nicotinamide 100, para-aminobenzoic acid 100, choline 100, and folic acid 100.

at the same dilutions of inositol as in the first titration. Titration 3 showed, for strains No. 1 and No. 5, that in the presence of adequate amounts of inositol the amount of growth is proportional to the amount of thiamine present with maximum growth occurring in tubes containing 0.2 μg. thiamine. It was noted that large amounts of thiamine—up to 5.0 mg.—had no inhibitory effect. The lower end point of the effect of thiamine occurred in concentrations of 0.02 and 0.04 μg. thiamine per 10 ml. asparagine broth. Beyond these there occurred only a small amount of growth, which could be accounted for by the inositol present. Titration 4 indicated, for strain No. 18, that in the presence of adequate amounts of both inositol and thiamine, pyridoxine was effective in concentrations as low as 0.02 μg. per 10 ml. asparagine broth. Strain No. 19, which had shown no vitamin requirements in previous studies, grew equally well in control tubes containing basal broth and in tubes containing the vitamins (Table 2).

TABLE 2

SMALLEST AMOUNTS OF THE ESSENTIAL VITAMINS WHICH WILL STIMULATE THE GROWTH OF SEVERAL STRAINS OF *T. faviforme* IN 10 ML. OF ASPARAGINE BASAL BROTH

Strain	Inositol	Thiamine (in the presence of 1000 μg. inositol)	Pyridoxine (in the presence of 1000 μg. inositol, and 50 μg. thiamine)
	μg.	μg.	μg.
No. 1 (album variety) Penna.	97.5	0.02	No effect
No. 5 (discoides variety) Penna.	12.2	0.04	No effect
No. 18 *T. discoides* Mackinnon Uruguay	48.0	No effect	0.02
No. 19 *T. album* (Baudet-Stuhmer) Holland	No effect	No effect	No effect

SUMMARY

1. As a result of cultural studies employing single spore strains of recently isolated, large-spored, ectothrix trichophytons of animal

origin, it is apparent that *Trichophyton album, T. discoides,* and *T. ochraceum* are variants of a single species. It is proposed that they be classified as *T. faviforme* (var. album, discoides, and ochraceum).

2. Growth of these strains is very slow and poor on Sabouraud's dextrose agar, and the small, glabrous colonies produced are composed of masses of poorly formed mycelium and numerous chlamydospores. Special enriched media are described which allow rapid development of these forms with the production of heavy, folded colonies with considerable powdery or downy white surface growth. On such media the mycelium is more regular in form and bears fewer chlamydospores. The aerial growth produces large numbers of microconidia, and, in most strains, macroconidia were also found. It would seem that the morphological characteristics usually described for this species on Sabouraud's dextrose agar are the results of inadequate nutrition.

3. Vitamin-free, asparagine basal media were used in studies of the vitamin requirements of freshly isolated strains as well as of stock strains of *T. faviforme.* Twenty-one recently isolated strains were shown to require i-inositol and molecular thiamine. One stock strain, *T. discoides* (Papegaay), had similar requirements, while two others, *T. album* (Baudet and Stuhmer) and *T. ochraceum* (Boedijn), were autotrophic for the vitamins. *T. discoides* (No. 688 Mackinnon), reported to require i-inositol, molecular thiamine, and pyridoxine by Robbins, Mackinnon, and Ma (11), was compared with these strains.

4. Rapid growth with characteristic spores was obtained on synthetic media to which had been added adequate amounts of the required vitamins. The amount of growth, however, was much less than that produced on a natural medium. The minimal effective quantities of the vitamins were determined by serial dilution methods.

5. Recent reports (6, 15, 16) indicate that *T. faviforme* is not an uncommon agent of ringworm in this country. It is prevalent particularly in cattle raising areas, and human infections can usually be traced to contacts with infected cattle.

6. In view of the fact that *T. faviforme* has requirements for

optimal growth which are not supplied by the usual cultural procedures and media, it is recommended that special vitamin-enriched media with added antibiotics, and incubation at 37° C., be used for attempted isolation of this fungus.

The author wishes to express her gratitude to Dr. Rhoda W. Benham of the Department of Dermatology, Columbia University, College of Physicians and Surgeons, for advice and assistance in this work.

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ILLUSTRATIONS

FIG. 5. *a, T. faviforme* (strain No. 11) which requires inositol and thiamine. Addition of pyridoxine or mixture of other vitamins does not change amount of growth; *b, T. discoides* (No. 688 Mackinnon) which requires pyridoxine in addition to thiamine and inositol; *c, T. faviforme* (strain No. 8) requires inositol and thiamine. The molecular thiamine cannot be replaced by thiazole, pyrimidine, a combination of these or oxalacetate.

FIG. 6. *a, T. faviforme* (strain No. 5) on basal agar with inositol and thiamine. Note large numbers of microconidia; *b, T. faviforme* (strain No. 8) on basal agar with inositol and thiamine. Note large numbers of macroconidia

THE NUTRITIONAL REQUIREMENTS OF EREMOTHECIUM ASHBYII GUILL.*

EUGENE L. DULANEY AND F. H. GRUTTER

In his descriptions of *Eremothecium ashbyii,* Guilliermond (**1, 2**) noted the production of large quantities of a pigment that colored the mycelium yellow and diffused into the culture medium. Within the cells, crystals of the yellow pigment were found in the vacuoles. In another publication, Guilliermond, Fontaine and Raffy (**3**) classified this pigment as a flavin and commented on its similarity to riboflavin. This flavin was later extracted and crystallized by Raffy and Mirimanoff (**4, 5**).

Although *E. ashbyii* has attained some industrial importance as a source of riboflavin, there have been few publications concerning the physiology of this species. One such paper is that of Schopfer (**6**) and it is this publication that we would like to refer to at this time.

Schopfer found that *E. ashbyii* would grow in natural media but not in a synthetic medium containing glucose 1%, glycine or asparagine 0.1%, magnesium sulfate 0.05% and acid potassium phosphate 0.15%. When various peptones were added singly to this medium, growth and riboflavin production occurred. The response varied with the peptone, and treatment of peptone with norite yielded a filtrate that did not stimulate growth when added to the synthetic medium noted above. When the norite-treated peptone plus biotin was added to the medium containing glucose and glycine, growth and riboflavin production occurred. In addition, vitamin B_1 and *i*-inositol were found to be complementary factors in that they intensified the effect of the biotin in this medium; however, they were not essential as was the biotin. It should be emphasized that no growth occurred in the synthetic medium supplemented only with the three vitamins or with the norite-treated

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* Contribution from the Research Laboratories of Merck & Co., Inc., Rahway, N. J.

peptone. In order to obtain growth, it was necessary to add both the norite-treated peptone and the vitamins, at least biotin, to the medium.

Recently we have been investigating the nutritional requirements of *E. ashbyii* and in particular its vitamin requirements. Although the experimental conditions used by us are not strictly the same as those used by Schopfer (6), it appears desirable to present some of our results at this time.

MATERIALS AND METHODS

We have used a basal medium containing glucose 1.0%, acid hydrolyzed casein 1.0%, K_2HPO_4, 0.2%, $MgSO_4 \cdot 7H_2O$ 0.1%, NaCl 0.02%, $CaCl_2$ 0.02%, $FeSO_4 \cdot 7H_2O$ 0.0005%, $ZnSO_4 \cdot 7H_2O$ 0.0005% and distilled water to volume. The casein used was the commercial product Labco sold by the Borden Co. as being vitamin-free. Before use it was acid hydrolyzed, neutralized with barium hydroxide and treated twice with norite. This basal medium was dispensed in 25 ml. amounts in 125 ml. Erlenmeyer flasks. All glassware used in these experiments was acid cleaned and thoroughly rinsed in distilled water. To this basal medium, biotin (0.04 μg./ml.), vitamin B_1 (0.1 μg./ml.) and *i*-inositol (0.2 mg./ml.) were added singly and in all possible combinations. The flasks were capped with heavy aluminum foil and sterilized by autoclaving at 15 lbs. pressure for 17 minutes. Sterile cotton plugs were used to replace the aluminum foil caps after autoclaving.

Two cultures of *E. ashbyii* were used in some of these experiments. One of these was obtained directly from Guilliermond and the other came from the Centraalbureau voor Schimmelcultures at Baarn. Schopfer notes in his 1944 publication that the culture used in his experiments came from Baarn. The 1943 Baarn catalogue lists only one culture of *E. ashbyii* and this is the culture included in some of our experiments. Both of these cultures have been in the Merck culture collection for several years. It should be emphasized, however, that there is no certainty that we have used the same strain of *E. ashbyii* that Schopfer used.

The inoculum for these experiments was grown in 25 ml.

amounts of yeast-extract glucose broth in 125 ml. Erlenmeyer flasks. After 48 hours growth on a rotary shaker, the mycelium was centrifuged and washed four times with sterile distilled water, then made up to one half the original volume with sterile distilled water. Three drops of this suspension were used to inoculate each flask. The incubation temperature for all experiments was 28° C. All flasks were incubated on rotary shakers moving at 220 rpm and describing a horizontal circle one and one half inches in diameter. In addition, replicate flasks of the treatments concerning the effects of vitamins were incubated statically.

The riboflavin content of the metabolism solutions was determined fluorometrically.

Three replicate flasks were used for each treatment and the experiments have been repeated several times.

RESULTS AND DISCUSSION

Comparable results have been obtained in experiments incubated statically and on rotary shakers. The results of a representative experiment concerning the effects of adding biotin, *i*-inositol and vitamin B_1 to the basal medium, singly and in combination, are shown in TABLE I. The culture of *E. ashbyii* used in this experiment was the one obtained from Guilliermond. The flasks were incubated on a shaker.

TABLE I

THE EFFECT ON RIBOFLAVIN PRODUCTION OF ADDING BIOTIN, VITAMIN B_1, AND *i*–INOSITOL, SINGLY AND IN COMBINATION, TO THE FERMENTATION MEDIUM

Treatment	Riboflavin broth potency µg./ml. after			Dry wt. mg./ml. after 7 days
	3	5	7 days	
Basal medium	5	6	8	
B.M. plus biotin	5	6	6	
B.M. plus vitamin B_1	9	8	11	
B.M. plus *i*-inositol	40	57	97	5.3
B.M. plus biotin and vitamin B_1	7	8	10	
B.M. plus biotin and *i*-inositol	61	67	86	4.2
B.M. plus vitamin B_1 and *i*-inositol	46	79	105	3.2
B.M. plus biotin, vitamin B_1 and *i*-inositol	73	69	82	4.3

Growth in the basal medium was very sparse when measured visually. There may have been some slight cell proliferation but it was so small that dry weight measurements were not made. In addition, biotin and vitamin B_1, when added singly or in combination to the basal medium, did not increase the amount of growth or riboflavin production over that obtained in the basal medium. In these treatments, growth, if it occurred, was so slight that it was not measured. There was, however, some riboflavin production in these treatments. A level of approximately 7 μg./ml.–10 μg./ml. was reached by the third day after which there was little increase. It may be noted that the dry weight of cells added to each flask as inoculum was approximately 1.5 mg. Apparently some stimulatory substances and possible intermediates in the biosynthesis of riboflavin were carried over with the inoculum in amounts sufficient to allow some cell proliferation as well as chemical transformation to riboflavin. When the small amount of mycelium in the flasks showing slight growth and low riboflavin levels was removed aseptically by centrifugation, washed with sterile distilled water and used to inoculate other flasks containing media with the same vitamin supplements, no observable growth or riboflavin production occurred.

Growth and riboflavin production, however, were obtained in the basal medium supplemented with *i*-inositol alone or in the media supplemented with any combination of vitamins that included *i*-inositol. The addition of biotin and vitamin B_1 to the basal medium containing *i*-inositol did not result in an increase in the maximum riboflavin level over that obtained in the basal medium containing *i*-inositol alone. There was, however, an apparent earlier increase in riboflavin level than occurred in the basal medium supplemented only with *i*-inositol.

In all of the treatments in which growth occurred the experiment was carried through three passages. Comparable results were obtained with the Baarn culture of *E. ashbyii*.

These results indicate that *E. ashbyii* has an absolute requirement for *i*-inositol. They do not, however, indicate an absolute requirement for biotin or vitamin B_1. There would seem to be a possibility that the hydrolyzed casein contained low levels of biotin even after the treatments given it. However, no biotin could be demonstrated by microbiological assay using an organism having a requirement for biotin. By the same method, vitamin B_1 could not be detected in the hydrolyzed casein. In addition, when L (−) proline, DL glutamic acid and L (+) arginine were substituted singly for the hydrolyzed casein in the basal medium, good growth and riboflavin production occurred if only *i*-inositol was used as a supplement. It is possible that the natural amino acids such as L (−) proline and L (+) arginine could be contaminated with biotin. The DL glutamic acid, however, was produced by organic synthesis and there is very little likelihood of its being contaminated with vitamins.

An observation may be made about the failure of Schopfer to obtain growth in his synthetic medium to which biotin, vitamin B_1 and *i*-inositol were added. This medium contained only glycine as a source of nitrogen. The two cultures of *E. ashbyii* used in our experiments will not use glycine as a sole source of nitrogen. It may be that the norite-treated peptone that had to be added to this medium containing these three vitamins in order to obtain growth was merely supplying an available source of nitrogen. When we substituted L (−) proline, L (+) arginine or DL glutamic acid for the glycine in Schopfer's synthetic medium, though at a higher level than 0.1%, growth and riboflavin production readily occurred if only *i*-inositol was added. No other absolute growth requirement could be demonstrated.

In all of the above experiments, the level of riboflavin produced is much lower than that obtained in more complex organic production media. These low levels of riboflavin are due, in part, to the low concentrations of carbon and nitrogen employed in these defined media. It may well be, however, that as yet unidentified factors exist that stimulate production of riboflavin by *E. ashbyii*. The following compounds, however, when added singly to the basal medium plus *i*-inositol did not induce any increase in riboflavin production: glutamine 5.0 µg./ml., pantothenic acid 1.0 µg./ml., vitamin B_{12} 0.001 µg./ml., nicotinic acid 10.0 µg./ml., pyridoxine 10.0 µg./ml., choline 5.0 µg./ml., para aminobenzoic acid 1.0 µg./ml. and folic acid 0.1 µg./ml.

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A NEW SPECIES OF GELASINOSPORA[1,2]

CONST. J. ALEXOPOULOS AND SUN HUANG SUNG

(WITH 6 FIGURES)

INTRODUCTION

The genus *Gelasinospora,* closely related to *Sordaria* and *Neurospora,* was erected by Dowding (1) in 1933 to include two species of Pyrenomycetes with dark, pitted ascospores, *Gelasinospora cerealis,* and *Gelasinospora tetrasperma,* described by her at that time. A third species, *Gelasinospora autosteira,* found growing on Spanish moss in Natchez, Mississippi, in March 1949, is now added.

Gelasinospora autosteira differs from *Gelasinospora tetrasperma* in that its asci are normally octosporous, from *Gelasinospora cerealis* in its somewhat larger perithecia and in the smaller size of its ascospores, and is unlike both species in that it is composed of two strains, self-sterile and inter-fertile, designated A and B.

SPECIES DIAGNOSIS

Gelasinospora autosteira spec. nov.

Mycelium ramosius, hyphae 3–14.6 μ diam.; perithecia superficialia, subglobosa, membranacea, 0.42–0.61 mm. diam., 0.65–0.71 mm. alta, atra, glabra, rostro cylindrico et prominente; asci octospori, aparaphysati, cylindrici, 14.1–18.3 μ × 218.6–244 μ; ascospori primo hyalini, deinde fusco-brunnei vel atri, opaci, faveolati, 10.6–14.1 μ × 16.7–26.5 μ, binucleati; conidia et spermatia incognita.

Thalli auto-incompatibiles; species consistens ex duabus lineis designatis A et B.

Melrose Estate, Natchez, Mississippi, U. S. A., 20 Martii, 1949.

Hab. in caulibus *Tillandsiae usneoidous.*

Mycelium profusely branched, hyphae 3–14.6 μ diam.; perithecia superficial, pyriform, membranous, 0.42–0.61 mm. diam., 0.65–

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[1] Contribution No. 50–3 from the Department of Botany and Plant Pathology, Michigan State College.

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0.71 mm. tall, black, glabrous; beak prominent, cylindric, characteristically bent or hooked at the tip; asci normally octosporous, cylindric, persistent, 14.1–18.3 μ × 218.6–244 μ; ascal walls thickened at the apex around a central canal which terminates in a round pore; paraphyses lacking; ascospores oval, hyaline and transparent when young, changing to yellow and finally to very dark brown or black, opaque, 10.6–14.1μ × 16.7–26.5 μ, binucleate at maturity; characteristic pits on ascospore walls best visible just before maturity of ascospores has been attained; conidia and spermatia unknown.

Thalli self-incompatible, species consisting of two inter-fertile strains designated A and B.

Collected at Melrose Estate, Natchez, Mississippi, U. S. A., on March 20, 1949, and developed to maturity in moist chamber.

On stems of *Tillandsia usneoides.* Specific epithet *autosteira* (*auto* = self + *steira* = fem., sterile) refers to the self sterility of thalli derived from the growth of single ascospores.

Portions of the type specimen deposited in the herbarium of Michigan State College, the New York Botanical Garden, and the British Museum.

Cultures of A and B strains deposited with the American Type Culture Collection, Washington, D. C., the Centraalbureau voor Schimmelcultures, Baarn, Holland, and the Commonwealth Mycological Institute, Kew, Surrey, England.

MATERIALS AND METHODS

The fungus was first isolated in early April 1949 from a specimen of *Tillandsia usneoides* collected three weeks earlier at Natchez, Mississippi, while the senior author was visiting the southern United States. The *Tillandsia* specimen was collected and placed in a large phial in the hope that Myxomycete fructifications might develop thereon in a moist chamber. Instead, perithecia of a Pyrenomycete (FIG. 5), clearly belonging to the genus *Gelasinospora,* were observed when the specimen was examined upon return to East Lansing.

The observations reported below are based on cultures derived from single ascospore isolations. The isolations were made with the aid of fine, glass needles which were used to isolate ascospores under the dissecting microscope at 36 or 72 magnifications. For serial isolations from a single ascus, the ascus was isolated on a sterile slide, transferred to corn meal agar in a Petri dish, and the ascospores pushed out in series. They were then transferred to separate Petri dishes and allowed to form colonies from which transfers were made to agar slants for stock cultures.

Three series of isolations were made. Series G–1, 2, 3, and 4 consisted of four monosporous isolates obtained from random isolations of ascospores which had been ejected from the perithecia in the moist chamber and had fallen on the filter paper on which the material was resting. From a number of ascospores placed on corn meal agar, only four germinated yielding the above series. Series GOa–1, 2, 3, 5, 6, 7, and 8 was obtained when eight ascospores were isolated in series from a single ascus on corn meal agar and heated at 60° C. for thirty minutes. All but No. 4 germinated and yielded stock cultures. Series Gt–1, 2, 3 and 4 was obtained from an ascus that contained only four ascospores near the upper portion. Although ordinarily tetrasporous asci also contain the remnants of the four aborted spores, in this one no aborted spores were evident. It is possible that four ascospores had escaped leaving the other four still in the ascus. All four of these ascospores germinated after thirty minutes in the drying oven at 60° C.

Difco corn meal agar alone or with 2% Difco malt extract added was used for the experiments reported below.

For comparison with *Gelasinospora cerealis,* the other octosporous species in this genus, a culture (Winnipeg isolation) obtained through the kindness of Dr. E. Silver Keeping was employed.

For staining ascospore nuclei, the propiono-carmine smear method, as described to the senior author by Dr. L. S. Olive and modified by the junior author, was used. The method consists, briefly, in fixing a block of agar bearing perithecia in a mixture of 3 parts absolute alcohol and 1 part propionic acid over night, transferring to 4% iron alum for ten minutes, washing with running water for about 4 hours, and crushing the perithecia on clean slides in propiono-carmine prepared in accordance with the directions given by Sass (**5**) for aceto-carmine, but substituting propionic acid for acetic acid. After the cover slip was in place, the mount was heated over a flame several times, sealed with glycerine jelly, and stored at room temperature for a few days, until the nuclei were well stained.

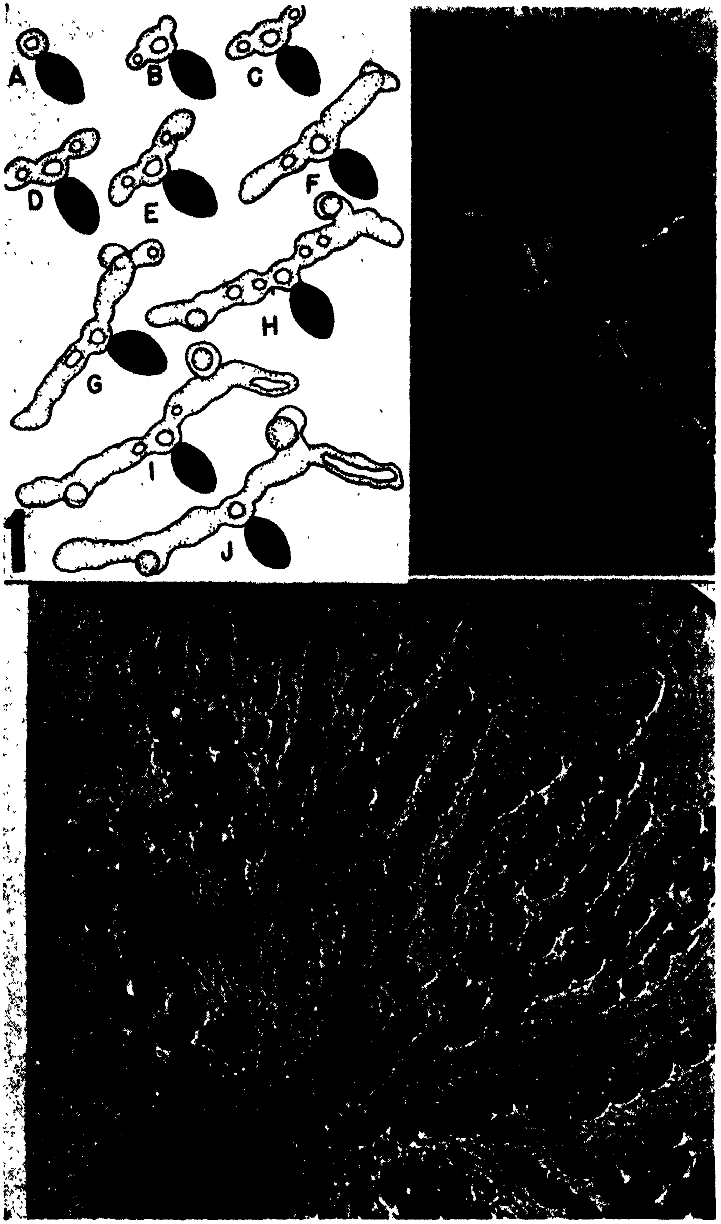
ASCOSPORE GERMINATION

The ability of the ascospores of *Gelasinospora autosteira* to germinate is apparently an inherited characteristic as it has been shown to be for *Neurospora* by Lindegren (4). Some ascospores will germinate much more readily than others and some will respond to heat treatment better than others. It appears also that the ascospores resulting from certain crosses germinate more easily than those from other crosses. These phases of the problem, however, have not been systematically investigated.

Dowding (1) reports 26% germination of unheated spores of *Gelasinospora cerealis* and *Gelasinospora tetrasperma.* Only 9% germination was obtained with *Gelasinospora autosteira* when 100 untreated spores, selected at random, were placed on corn meal agar. Treatment with biotin, freezing, placing at 58° C. for 30 minutes, and the combination of freezing and heating, failed to increase the percentage of germination in this experiment. In view of the above, it was particularly fortunate that seven of the eight spores in series GOa and all four of the spores in series Gta germinated.

In subsequent experiments, the details of which will be reported in a later paper, it was discovered that the method successfully employed by Dodge (3) for various Ascobolaceae considerably increased the percentage of spore germination in *Gelasinospora autosteira.* This method, as applied here, consists of placing spores, resting on the surface of corn meal agar, in a drying oven and slowly raising the temperature so that it reaches 70° C. in about 30 minutes. The heat is then turned off and the spores are permitted to remain in the oven until the temperature falls to 35° C. or below.

Ascospore germination on corn meal agar at room temperature begins with the formation of a vesicle four and one-half to five hours after planting. Growth is generally rapid after this and the entire surface of the agar in a Petri dish is often covered in about



FIGS. 1–3. A new species of *Gelasinospora*.

three days from the growth of a single ascospore. Figure 1, A–J, shows various stages in the germination of an ascospore. Such germination is very similar to that described by Dowding (1) for *Gelasinospora tetrasperma.*

MYCELIAL CHARACTERS

The mycelium of *Gelasinospora autosteira,* as it develops on corn meal agar, consists of rapidly growing hyphae of various thicknesses. The majority of the hyphae in a mature colony are about 4 μ in diameter, and irregularly septate, their cells varying considerably in length. Larger hyphae of an average diameter of 8 μ constitute the main strands of the colony. The septa in these are placed more regularly, the average length of the cells being somewhere between 25 and 35 μ. Giant hyphae are occasionally seen measuring 14.5 μ in diameter. The hyphal cells are probably coenocytic as they have been shown to be in *Gelasinospora tetrasperma* by Dowding and Buller (2). The mycelium was stained in 0.05% Heidenhain's haematoxylin after being fixed in Duggar's Gilson's fluid to which a few urea crystals had been added, in accordance with Dowding and Buller's recommendation. A large number of what appeared to be nuclei were stained in each cell, but these structures were so small that it was impossible to identify them with certainty.

The fungal colony is colorless at first, but in most monosporous isolates under study an over all brown color begins to manifest itself when the colony is about a week old. The intensity of the color varies with the isolate and may be linked with the compatibility factor according to present indications. On agar containing malt extract, the brown color becomes very dark.

Aerial mycelium is produced abundantly in media containing malt extract. It covers the colony completely and grows up the sides of the Petri dish and in columns from the surface of the agar, sealing the cover to the bottom of the dish. Abundant aerial mycelium often exhibits a pink color.

On corn meal agar, some aerial mycelium is also produced, generally in small tufts over the surface of the agar. Corn meal agar is preferable for some types of study and is now being used by the writers almost exclusively for growing this fungus because of its tendency to suppress formation of aerial mycelium, thereby making archicarps and perithecia more easily visible.

PERITHECIAL PRODUCTION

The ascocarps of *Gelasinospora autosteira* are pyriform, black perithecia with a prominent, hooked beak. On the natural host the perithecia are glabrous, but in culture the perithecial body is covered with many gray hairs, the beak alone being glabrous. In size, the perithecia are somewhat greater in diameter than those of *Gelasinospora cerealis,* measuring 0.42–0.61 mm.

Isolates G–1, G–2, G–3, and G–4, resulting from single ascospores selected at random, were grown singly and in all possible combinations in Petri dishes, on corn meal agar with 2% malt extract. None of the single isolates yielded perithecia, nor did the G–1 × G–2, and the G–3 × G–4 crosses. However, when G–1 and G–3, or G–2 and G–4 isolates were grown together, clusters of perithecia developed near the edges of the Petri dishes on the line of contact between the two mycelia. This experiment was repeated several times with the same results. It was concluded, therefore, that this fungus consists of two self-incompatible strains and that isolates G–1 and G–2 were of one strain while G–3 and G–4 were of the other. This is in contrast to the condition found by Dowding (**1**) in the other two *Gelasinospora* species which are both homothallic except for the self-incompatible strains derived from dwarf spores of *Gelasinospora tetrasperma.*

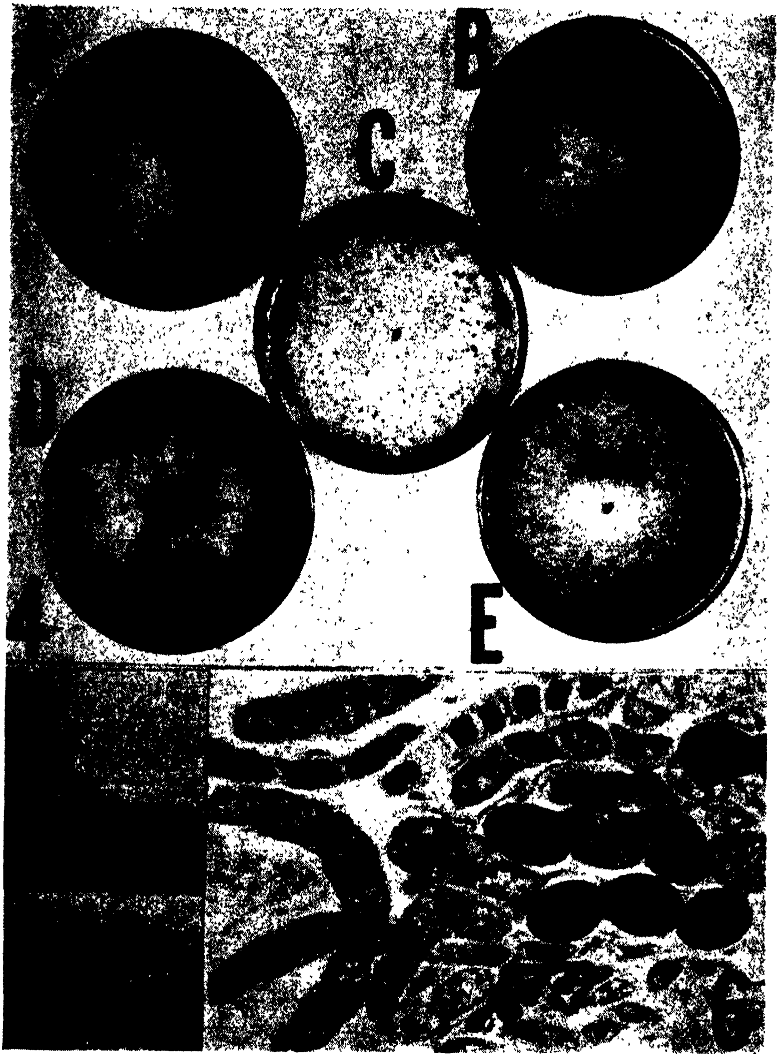
Isolates GOa–1, GOa–2, GOa–3, GOa–5, GOa–6, GOa–7, and GOa–8, all obtained from a single ascus, were similarly tested as single colonies and in all possible combinations. None of the single isolates yielded perithecia, nor did any combination between the first three or the last four isolates in the series. Some single colonies produced many minute archicarps, just visible to the naked eye, which remained undeveloped. All combinations between any one of the first three and any one of the last four yielded numerous perithecia, generally confined to the edge of the Petri dish in the region where the two mycelia met. This experience, repeated several times with the same results, constitutes further evidence that

Gelasinospora autosteira is not homothallic. It is more than probable that had GOa–4 germinated it would have been found to belong to the same strain as GOa–1, 2, and 3, the compatibility factor having segregated in this ascus in the first division.

A further test was made with Gta–1, 2, 3, and 4, isolated from an ascus containing only four ascospores. These were grown singly and in all possible combinations with no perithecia resulting in any case. If the assumption made earlier is correct, that the first four ascospores of this ascus had already escaped at the time of isolation, the result of these crosses would indicate that the compatibility factor segregated in the first nuclear division in this ascus as well as in the one which yielded the GOa series.

In *Gelasinospora autosteira*, as in *Gelasinospora tetrasperma* (2), nuclei from the A and B strains probably come together by means of somatogamy, no spermatia or conidia having been found in any of the three known species in this genus. When two compatible mycelia are mated in a Petri dish, the perithecia which are formed are generally located at some point or points of contact between the two colonies, or, in some cases, all along the line of contact (FIG. 4A). Perithecial development is often confined to one or two small areas at the margin of the dish on the line of contact between the two mycelia. This may be a response to the greater oxygen supply at the margin of the dish. In some cases no more perithecia develop, but in others, a second crop matures nearer the center of the dish following more or less the line of contact of the two mycelia (FIG. 4B). Dowding and Buller (2) state that *Gelasinospora tetrasperma* will not form perithecia in sealed Petri dishes. They found it necessary to grow the fungus in open Petri dishes in order to induce perithecial formation. Although *Gelasinospora autosteira* appears to prefer an abundance of oxygen for fruiting, as evidenced by the location of perithecia near the margin of the Petri dish when aerial mycelium is very abundant, the fungus is apparently not as sensitive to the amount of oxygen present as is *Gelasinospora tetrasperma* since it never fails to produce perithecia in closed Petri dishes, other conditions being favorable.

At room temperature, near a window, but without the aid of artificial light at any time, perithecia become clearly visible in about



FIGS. 4–6. A new species of *Gelasinospora.*

14 days after planting. Archicarps, when formed, usually appear in four to five days after planting and are barely visible to the unaided eye. The time of perithecial development, however, varies considerably and may be correlated with certain parental crosses and environmental conditions, as may also be the number of peri-

thecia formed, and their distribution in a Petri dish. These phases of the problem are under investigation.

Ascospores begin to mature about one month after planting. Forcible ejection of ascospores begins about a week after the first ascospores have matured as evidenced by their dark color.

Isolates which have been kept in culture by mycelial transfers for a few months lose their ability to produce perithecia altogether, and fertile strains may be reestablished only by germinating more ascospores. It is therefore advisable to have mixed cultures with perithecia always on hand if one is to do any considerable work with this fungus.

ASCI AND ASCOSPORES

Asci are produced abundantly in the perithecia of *Gelasinospora autosteira.* They are long and cylindrical. When pressed out of the perithecium under a cover slip, they spread out from a common center in a typical fashion (FIG. 3). There are no paraphyses. The asci are normally octosporous, but spore abortion is common. Asci with 3, 4, 5, 6, or 7 mature spores, the remaining aborted, can be found in almost any perithecium, but usually when abortion occurs, four spores abort and four develop.

Although there is some variation in the size of the ascospores within a single perithecium and a single ascus, no dwarf spores or giant spores have been found in this species. The characteristic pits of the ascospore walls which place this fungus in the genus *Gelasinospora,* can be seen best just before the spores mature while they are changing from yellow to light brown in color (FIG. 6). In the mature spores the wall is so opaque that the pits are virtually invisible.

The ascospores are considerably smaller than those of *Gelasinospora cerealis,* the other species with octosporous asci in this genus. No apiculate spores such as found by Dowding (1) in *Gelasinospora cerealis* have been found in the new species.

The ascospores show two nuclei when stained with propiono-carmine when still young (FIG. 6). Young ascospores with single nuclei also have been found. The nuclear situation appears to be similar to that in the other two species of this genus, the two nuclei in the mature spore probably being sister nuclei. Extensive cytological investigations have not been carried out as yet.

ACKNOWLEDGMENTS

We wish to express our sincere appreciation to the All College Research Committee of Michigan State College, for a grant, a portion of which was used to support this investigation; to Dr. E. Silver Keeping for supplying cultures of *Gelasinospora cerealis* and *Gelasinospora tetrasperma* for comparative studies; to Dr. L. S. Olive for helpful suggestions on the use of the propiono-carmine smear technique, and to Drs. B. O. Dodge and E. A. Bessey for valuable suggestions, the former through correspondence, the latter on his frequent visits to our laboratory. Thanks are also due to Dr. E. A. Bessey for criticizing the manuscript. Our gratitude also goes to Dr. William M. Seaman of the Michigan State College Department of Foreign Languages for correcting the Latin description and for his helpful suggestions in the selection of some descriptive words.

After this article was submitted for publication, the December 1949 number of the Supplément Colonial à la Revue de Mycologie was received. In an article entitled Quelques Ascomycètes du Congo, Dr. and Mme. Claude Moreau describe and figure (pp. 53–55) *Gelasinospora calospora* (Mouton) nov. comb. This is presumably a homothallic species which closely resembles our fungus in its morphology.

Correspondence with Dr. Roy F. Cain of the University of Toronto has also revealed that he has an article in press (Canadian Journal of Research) in which *Gelasinospora adjuncta,* consisting of two strains, is being described.

A comparative study of these organisms with ours is necessary to establish the validity of *Gelasinospora autosteira.*

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EXPLANATION OF FIGURES

FIG. 1. Ten stages in the germination of an ascospore, drawn at approximately half-hour intervals as follows: A—3:30, B—4:00, C—4:30, D—5:00, E—5:30, F—7:05, G—7:40, H—8:15, I—8:40, J—9:12. All camera lucida (× 375).

FIG. 2. Photomicrograph of ascospore 22 hours after planting (× 150).

FIG. 3. Photomicrograph of ascospores within asci, the latter spread out after crushing a perithecium under a cover slip. Note prominent ascal pores, aborted spores (× 250).

FIG. 4. Corn meal agar cultures of: *A. Gelasinospora autosteira* A and B strains with perithecia produced all along the line of contact between the two mycelia; *B.* A and B strains with perithecia produced first in two groups near the margin of the Petri dish at the point of contact of the two mycelia, and subsequently scattered between these two points; *C. Gelasinospora cerealis* showing numerous perithecia produced by a monosporous culture; *D. Gelasinospora autosteira,* producing no perithecia when two monosporous isolates of the same strain are grown together; *E.* Monosporous isolate of *Gelasinospora autosteira* producing no perithecia. All cultures of the same age, one month old.

FIG. 5. Perithecium of *Gelasinospora autosteira* on stem of *Tillandsia* (× 100).

FIG. 6. Ascospores of *Gelasinospora autosteira* at various stages of maturity stained with propiono-carmine. On the left, several ascospores showing two nuclei each. Lower margin, one ascospore with a single nucleus. On the right, ascospores with pitted walls (× 500).

URNULA CRATERIUM IS POSSIBLY THE PERFECT STAGE OF STRUMELLA CORYNEOIDEA

Ross W. Davidson [1]

(WITH 3 FIGURES)

In the Civilian Conservation Corps program in timber stand improvement work, which began in 1933, the Division of Forest Pathology became increasingly interested in cankers of hardwoods. The interest at that time was mainly from the standpoint of formulating methods of disease control. Cultures were obtained of a number of the more important canker fungi such as species of *Strumella, Nectria,* and *Eutypella* and some have been maintained in the stock culture collection of the Division of Forest Pathology at Beltsville, Maryland, since that time. *Strumella* canker (caused by *S. coryneoidea* Sacc. and Wint.) has long been regarded as one of the more important defects of species of oak and several other hardwoods (**1**, **3**, **4**, **6**, **8**). The perfect stage of the fungus causing this canker was not known but has been the subject of speculation among forest pathologists. The perfect stage has not been taken into consideration in formulating control practices and this may be illustrated by the following quotation from Boyce (2): "If cankered living trees with dead or likely-to-be-killed branches or sprouts adjacent to cankers cannot be removed, the branches or sprouts should be knocked off. . . . Furthermore, all standing dead trees with bark on should be knocked down." This was based on the observation that conidia of the fungus develop in abundance on infected dead standing trees although it has not been demonstrated that conidia are the source of infections. In fact, none of the investigators has been able to germinate conidia on artificial media (**1**, **6**, **8**).

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EVIDENCE OF A PERFECT STAGE OF STRUMELLA

In December 1948, peculiar fingerlike fungus growths (FIG. 1A) on an oak log were called to the attention of the writer.[2] These were on oak logs about 5 to 6 inches in diameter which had been cut during 1946 in clearing an area to be used for a golf course near Fairfax, Virginia. The growths were quite numerous and occurred on the under side of the logs where they had been in contact with the damp soil or leaf litter. They were dark gray to black, with a white powdery outer surface, about one inch long, cylindrical, rounded at the tip, and occasionally slightly enlarged at the tip end. They were about the diameter of a pencil. On sectioning they were found to be hollow above and with an immature hymenial layer on the inside, suggesting an immature discomycete. The bases of the immature fruiting bodies were surrounded by a black hairlike mass of mycelium (FIG. 1A).

Isolations from the inner bark tissue on which the fungus was growing yielded in 10 cases out of 12 what appeared to be cultures of *Strumella coryneoidea,* as shown by comparison with stock cultures of *Strumella* from oak cankers that had been maintained in the culture collection for about 15 years.

Additional sections of oak logs containing immature fruiting bodies of the fungus were collected near Fairfax, Virginia, in February 1949, and kept outdoors. On March 31, 1949, several of the immature sporophores had enlarged at the tip and started to split open in a stellate manner (FIG. 1B). No mature asci were present on this date and they did not mature when placed in a damp chamber in the laboratory for further development (FIG. 1C). However, pure cultures were readily obtained by picking out small bits of the hymenial tissue.

On April 18, 1949, several opened apothecia were again brought into the laboratory, but asci were immature, showing only in a few cases eight differentiated nuclei. On April 25, two apothecia were collected from the logs and taken to the laboratory. These contained a few apparently mature ascospores so that the sporophores were definitely identified as *Urnula craterium* (Schw.) Fr. On

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[2] Mr. Walter Roney, formerly of the Division of Forest Pathology, called attention to this fungus and assisted in collecting the material used in this study.

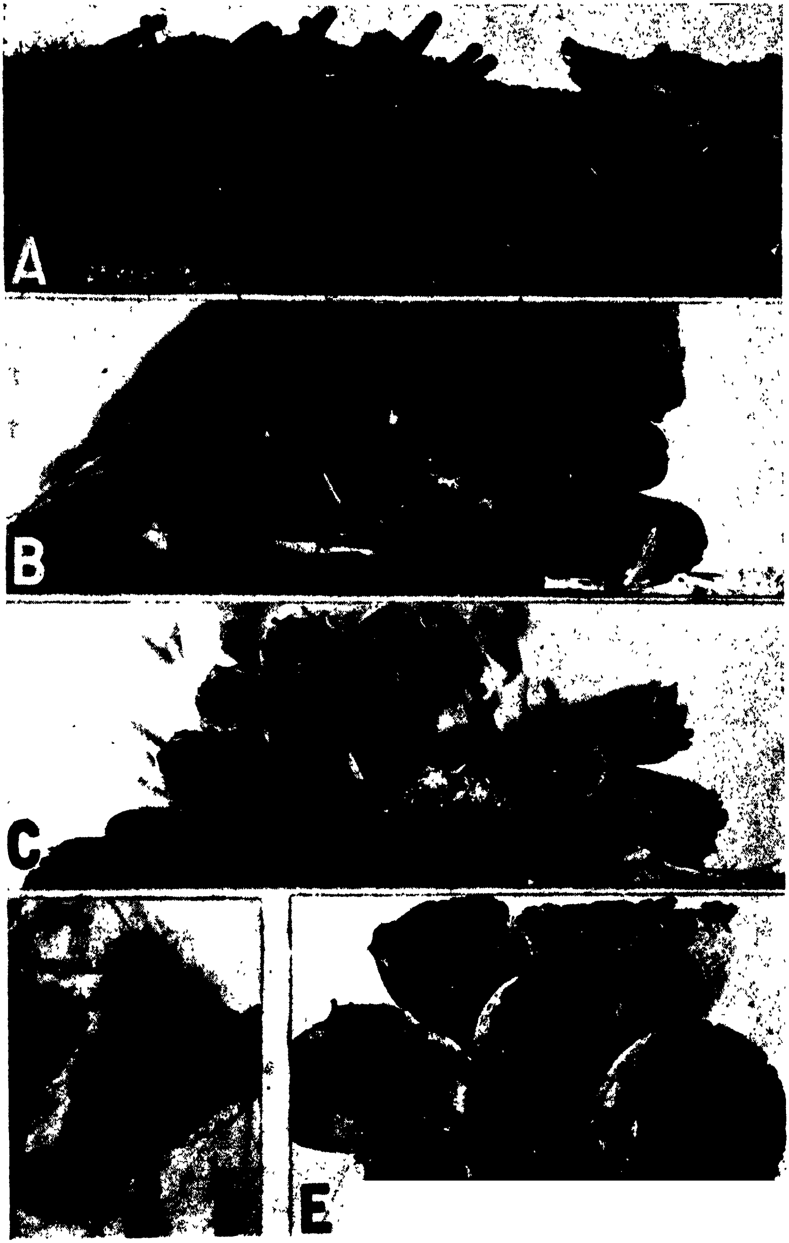

FIG. 1. Developmental stages of *Urnula craterium*.

April 26, two apothecia were quite wide open (FIG. 1D), and a deposit of ascospores was obtained. Cultures from the ascospores were made on malt and cornmeal agar media (FIG. 1D).

Within the following few days in late April, additional apothecia were collected in mature condition (FIG. 1E). When placed over Petri dishes containing cornmeal agar, ascospores were deposited in abundance. The spores were sometimes shot out in scattered groups of eight or only one or two spores at a time. Cultures were readily obtained.

Germination occurred from one end in 3 to 4 hours in 90 to 100 per cent of the ascospores shot from the apothecium. Sometimes a second germ tube grew from the opposite end within about 24 hours after the spores had been deposited on agar. Subsequent growth of germ tubes was very rapid. In a 24-hour period the germ tubes were many times the length of the spore.

CULTURES OF URNULA CRATERIUM AND STRUMELLA

Cultures of *Strumella coryneoidea* as obtained from cankers are rather distinctive and differ considerably from cultures of any other fungus which the writer has studied. They grow rapidly, forming a light gray dense mat on malt agar. Old cultures develop dark, sometimes black areas. In test tube cultures rather dense black masses of fungus tissue develop between the glass and the agar substratum (FIG. 2B), as described by Bidwell and Bramble (1). These appear to be somewhat comparable to the masses of fungus tissue forming the pustules that break through the bark—serving as pressure pads. Those that develop in test tubes are composed of masses of coiled segments of dark black, encrusted hyphae 3 to 5 μ in diameter.

Cultures obtained from the bark on which immature fruit bodies of *Urnula* were growing and tissue isolations from the nearly mature sporophores were similar to cultures of *Strumella* in growth rate on malt agar, malt plus gallic acid, and malt plus tannic acid. They were slightly more zonate and the mycelial mat on malt was a little darker than those of the old stock cultures. Cultures from single ascospores were lighter and less zonate, or nearly the same as the cultures of *Strumella*. This variation in cultures from dif-

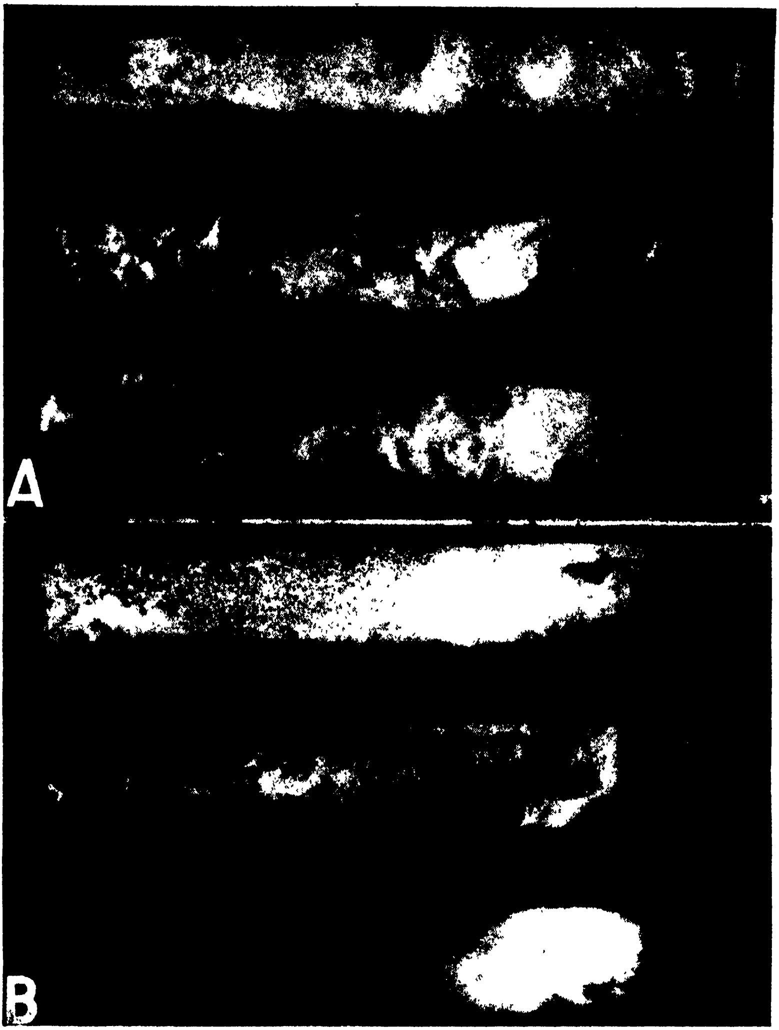

FIG. 2. Cultures of *Urnula* and *Strumella.*

ferent sources is believed to be no greater than would be expected with cultures of a single species from different sources. The *Urnula* cultures also developed the black stromatic masses in test tube cultures (FIG. 2A).

The growth and reactions of *Strumella* on media containing gallic and tannic acid were recorded in a previous paper (5). The growth and reaction of cultures of *Urnula craterium* on these media are very similar. Growth rate of both fungi on two per cent malt agar at ordinary room temperature of 24° C. is about 50–60 mm. in diameter, in one week. On gallic acid medium under similar conditions it is 0–20 mm., on tannic acid about 20–30 mm. Both fungi give a strong reaction on gallic and tannic acid media.

Conidia of *Strumella* have never been observed to develop in pure cultures so none would be expected under ordinary culture conditions. They might be induced to form if different types of substrata were used.

URNULA IN AREAS WHERE STRUMELLA CANKERS ARE NUMEROUS

The only sporophores observed in the spring of 1949 were all from oak logs collected at the one place near Fairfax, Virginia. A search at other places near the District of Columbia, especially at Beltsville, Maryland, failed to locate any additional specimens. *Strumella* cankers are not known to be common in this general area.

On December 8, 1949, additional material of *Urnula* was collected on a canker control plot near Oakland, Maryland. In this area *Strumella* cankers are abundant on oak reproduction. Cankered trunks that had been felled or that had broken over and remained on the ground for several years, contained abundant young fruits of *Urnula*. These were about the same as those collected near Fairfax, Virginia, but were mostly younger and somewhat more cone-shaped, with a broad base, and tapering to a rounded tip (FIG. 3). From this material it is evident that the fruit bodies start to develop in the fall, probably in October or early November. They appear to develop only on trunks that have been lying on the damp ground for some time. It is believed that *Urnula craterium* is a common fungus in other localities where *Strumella* cankers are prevalent.

In a recent paper Nannfeldt (7) described a new species, *Urnula hiemalis*, which has slightly smaller ascospores and shorter stipes than *U. craterium* and grows on grassy slopes or sparse lawn, apparently not attached to sticks. The new species develops during the winter in which respect it is probably similar in habit to *U. craterium* except that the apothecia may open up earlier. Both

FIG. 3. Young sporophores of *Urnula* photographed on Dec. 9, 1949.

species have the coarse black hairlike mat at their base. *U. craterium* is reported as very rare in Europe.

CONCLUSIONS

Present observations and studies strongly suggest that *Urnula craterium* is the perfect stage of the fungus causing *Strumella* cankers. Cultures of *U. craterium* have been compared with several cultures of *Strumella* which were originally isolated from cankers. These cultures were found to be very similar. Sporophores of *Urnula* start to develop in the fall (October or November) and mature by the latter part of April or the first part of May. Ascospores germinate in a few hours when deposited on nutrient agar media. Sporophores of the perfect stage have been observed to develop only on wood in contact with the damp soil and leaf mold.

They were found in abundance in an area where *Strumella* cankers are common.

This evidence suggests that in timber stand improvement work designed to reduce canker incidence, trees with *Strumella* cankers should not be cut down and left on the ground.

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DESCRIPTION OF ILLUSTRATIONS

FIG. 1. *Urnula craterium* in several stages of development. A. Young sporophores collected in December 1948. B. Sporophores, collected March 31, 1949, just starting to split open at the tip. C. The same sporophores shown in B, after being held in the laboratory for several days. Asci were present but ascospores had not developed. D. Sporophores collected April 25, 1949. A few mature ascospores were present at that date. E. Mature sporophores collected April 28, 1949.

FIG. 2. A. Cultures of *Urnula craterium* at different stages of development. B. Stock cultures of *Strumella coryneoidea* at different stages of development, isolated from cankers.

FIG. 3. Young sporophores photographed December 9, 1949, in fresh condition on cankered oak stem that had been lying on the ground, near Oakland, Maryland.

NEW AND NOTEWORTHY LICHENS FROM MT. RAINIER NATIONAL PARK

HENRY A. IMSHAUG

(WITH 2 FIGURES)

During the summer of 1948 the writer was fortunate in having the opportunity to assist Dr. A. H. Smith in a mycological survey of Mt. Rainier National Park, in the state of Washington. A number of papers dealing with the new and noteworthy fungi collected there have already appeared, and others are in preparation. The purpose of this paper is to discuss some of the new or interesting macrolichens. The microlichens will be considered at a later date.

I wish to take this opportunity to express my appreciation to Dr. E. B. Mains, director of the University of Michigan Herbarium, for making this survey possible, as well as for reading the manuscript, and also to Dr. A. H. Smith and J. Hedrick Jones for their advice and suggestions in the course of the collection and determination of the specimens and preparation of the manuscript. I am especially indebted to T. E. Hasselrot, Veli Räsänen, C. W. Dodge, and Ove Almborn for the loan of type material and other critical specimens in the course of this study.

1. A CORTICOLOUS *Cetraria* IN THE *islandica*-GROUP

This *Cetraria* was very abundant on the lower branches of small, thicket-forming shrubs, *e.g.*, *Rhododendron albiflorum* and species of *Vaccinium*. Also, it grew equally well on the soil around the bushes, especially in the more open areas at the higher elevations. In the literature this plant has been described both as *C. islandica* mod. *arborialis* and *C. tenuifolia* var. *pseudoislandica* f. *septentrionalis*.

Although this lichen differs markedly from both *C. islandica* and *C. crispa* (= *C. tenuifolia*), previous workers have considered it to be a modification resulting from its corticolous habitat. Th.

Fries (1871, p. 98) observed that small forms of *C. islandica* were sometimes to be found on wood and so little significance was attached to the American specimens.

The material found on Mt. Rainier was fruiting abundantly and large collections were made. The apothecia are marginal, at times almost stipitate, rather than subterminal on dilated apices as in *C. islandica* and *C. crispa.* The apothecial margins are not ciliate. These characters, together with the almost plane, nitidous laciniae, the complete lack of laminal pseudocyphellae, and the chemical reactions (Pd—, I—), clearly show, in the writer's opinion, that this plant cannot be placed as a variety or form under either *C. islandica* or *C. crispa.* Also, the Mt. Rainier material clearly shows that these differences are not due to a corticolous habitat, *i.e.*, the lichen was equally abundant on shrubbery and on the ground, without any apparent morphological change. When lignicolous, however, it is fastened to the substratum by black, adhesive discs which develop from the tips or the margins of the laciniae which come in contact with the bark. Growth of the lichen creates a tension on the laciniae fastened to the bark and eventually the adhesive discs are pulled away from the substratum. These discs persist on the elevated laciniae and are characteristic of the species when growing on bark.

It was, of course, desirable to compare this material with the Swedish specimens of *C. islandica* mentioned by Th. Fries and others as occasionally growing on wood. For this purpose, Dr. T. E. Hasselrot, of the Riksmuseum, Stockholm, was kind enough to examine the more than 900 envelopes of this group in that herbarium and to forward for study the twenty-five corticolous specimens, collected by Efr. Eriksson, which he found. Most were on stems of *Juniperus communis,* but some were from spruce and pine. Twenty-one were typical *C. islandica,* whereas four were typical *C. crispa.* In addition, the writer was able to examine a specimen of *C. islandica* f. *rhododendri* (Britzelmayr, Lichenes exs. aus der Flora von Augsburg, No. 633, Farlow Herbarium, Harvard University), which proved to be typical *C. islandica.* The Mt. Rainier material showed no similarity to any of these corticolous specimens.

It may be added that instances of *C. crispa* growing on wood without any apparent morphological change in the thallus are not entirely unknown in North America, as the writer has collected specimens on low juniper branches and on wood débris along the shores of Lake Michigan and Lake Huron in the state of Michigan.

In view of the information presented here it is concluded that both *C. islandica* and *C. crispa* are able to grow on small stems and branches, stumps, and old wood, without any apparent morphological change, and that the lichen known as *C. islandica arborialis,* which has been viewed as a modification of *C. islandica* due to its corticolous habitat, differs from both *C. islandica* and *C. crispa* by a combination of characters such as the position of apothecia, the lack of laminal pseudocyphellae, and the chemical reactions. Consequently, it cannot be considered as a variety or form under either of these species.

The question of nomenclature merits special consideration. The first published name was *C. islandica* modification *arborialis* (conditional nomination) Merrill. In addition to the peculiar designation, the following facts should be noted: 1) no type specimen was cited; 2) the description was based on two different collections, representing two distinct species, as shown by Howe (**1915**). Following the International Rules, Merrill's publication of the epithet *arborialis* cannot be considered valid.

The second publication of this name was by Howe (**1915**). Again, several facts may be noted: 1) the composite nature of Merrill's description was corrected; 2) a type specimen was designated; 3) the rank given to this name remained that of modification. Howe's use of the term modification may be explained by the following quotation (**1915**, p. 219): "It is evidently a modification as Mr. Merrill called it, caused by the corticoline substrata, a more or less accidental result of environment. . . ." He concludes by saying "further material of this interesting modification may prove to argue its acceptance as a variety or even species." In the writer's opinion this cannot constitute valid publication as Article 37 of the International Rules (Camp *et al.,* **1947**) says "A name of a taxonomic group is not validly published unless it is definitely accepted by the author who published it. A name proposed provisionally (nomen provisorium) in anticipation of the eventual acceptance of the group or of a particular circumscription, position or rank of a given group, or merely mentioned incidentally is not published."

The use of the combination *C. islandica* var. *arborialis* by Zahlbruckner (**1930**, p. 333) without any comment or without citing a particular description does not seem valid in view of the fact that Merrill's original description was a composite, based on two distinct species.

Finally, the epithet *septentrionalis*, used at the form level by Räsänen, is not available for use at the species level, due to the existence of *Cetraria septentrionalis* (Nyl.) Almqu., a synonym of *C. chrysantha* Tuck.

In view of these considerations it is necessary to propose a new name for this species. One which indicates its subalpine habitat is deemed suitable.

Cetraria subalpina sp. nov. FIG. 1

C. islandica m. *arborialis* Merrill, Bryologist **9** (1) : 4. 1906.
C. islandica var. *arborialis* Zahlbr., Cat. Lich. Univ. **6**: 333. 1930.
C. islandica var. *pseudoislandica* f. *septentrionalis* Räsänen, Ann. Mo. Bot. Gard. **20**: 9. 1933 (type seen).

Thallus laciniato-lobatus, laciniis cartilagineis, procumbens ad erectis, obtortis, irregulariter ramosis, superne et subtus glaber, nitidus. Margo laciniarum fere spinis. Color pallidus, pallide castaneo-umbrinus, olivaceo-umbrinus vel subfuscus. Pseudocyphellae longae et angustae, in marginibus. Rhizinae desunt. Soredia et isidia desunt. Apothecia 2.5 mm. lata, in marginibus laciniarum; margo apotheciarum non ciliatus. Sporae subglobosae, diam. 4.3–6.4 μ. Thallus extus et intus K—, K(C)—, C—, J—, Pd—. Specimen typicum in ramis, 6000 ft., Mt. Rainier National Park, Washington, U. S. A., Imshaug 1876, in Herb. Mich.

Thallus composed of densely tangled, twisted, irregularly divided, prostrate to erect laciniae. Laciniae cartilaginous, glabrous, nitidous; plane or with prominent central groove, occasionally with two grooves separated by a central ridge, but not canaliculate-connivent as in *C. crispa;* never expanded, main branches 4.0–6.5 mm. across, tips 1.0–1.5 mm. across; margins ± spinulose. Color very variable, pallid, greenish-olivaceous, olivaceous-fuscescent, earth-brown, rich chestnut brown, or combinations of these; lower

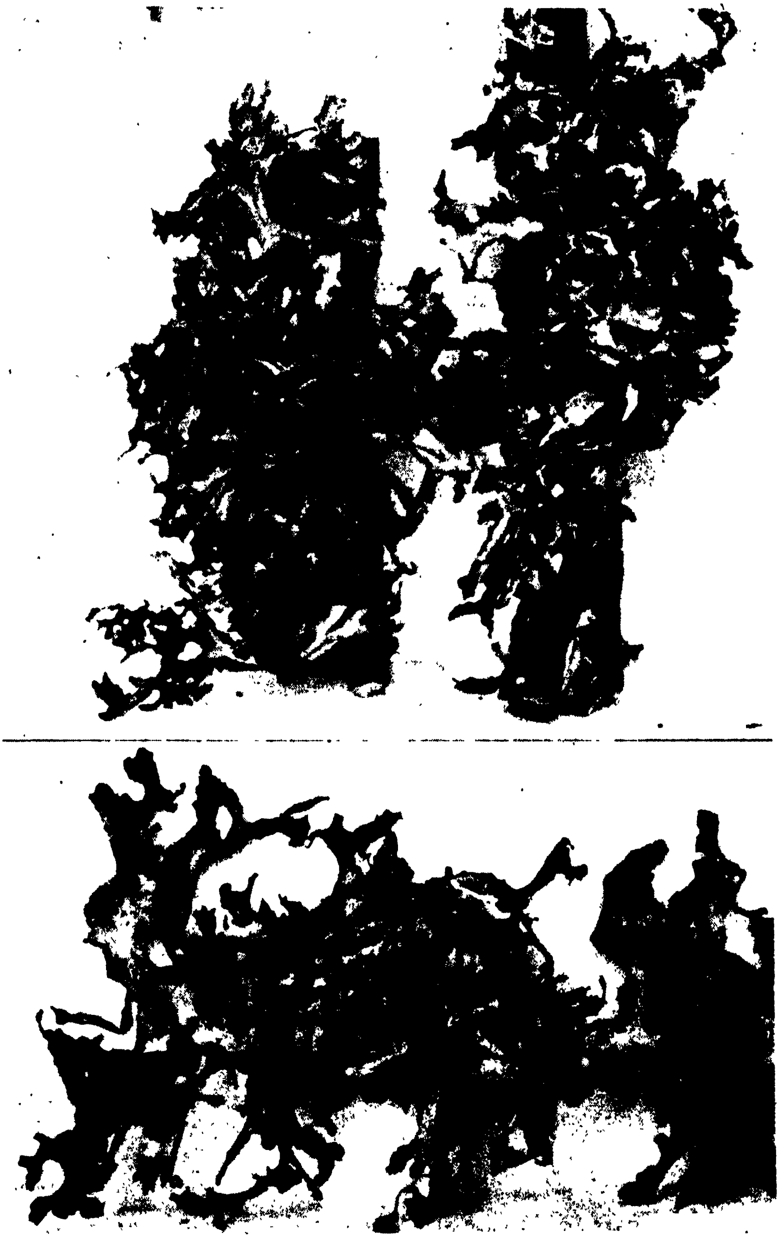

FIG. 1. *Cetraria subalpina.* Upper, corticolous; lower, terricolous.

surface generally lighter than upper surface, often pallid. Pseudocyphellae inconspicuous, marginal, long and narrow. Rhizoids none. Soredia and isidia none. Apothecia marginal, originating from upper surface of laciniae. Disc buff to light brown, 2–5 mm. across. Margin at first prominent, inrolled, at length disappearing; ± scalloped; not ciliate.

Thallus 150–220 μ thick; upper cortex scleroplectynchymatic, uncolored, 25–32 μ, with cuticle c. 3.2 μ thick; algae bright green, spherical, 8–11 μ in diam., in ± continuous zone 30–45 μ thick, also scattered in medulla; medulla of loose, pachydermatous hyphae, colorless, white in reflected light, 50–70 μ thick; lower cortex similar to upper cortex. Hymenium colorless, yellow-fuscescent above, 50–80 μ; asci subcylindric to narrowly clavate, 35–52 × 8.4–12.7 μ; spores 8, subspherical to irregular, 4.3–6.4 μ across; paraphyses simple, unbranched, c. 2.2 μ across, apices not capitate; hypothecium colorless, 68–82 μ.

Chemical reactions: Thallus Pd—, J—, K—, K(C)—, C—; medulla Pd—, K—, K(C)—, C—, J—; asci J + (blue) above.

Habitat: On branches of small shrubs, *e.g.*, *Vaccinium* spp. and *Rhododendron albiflorum*, and on the soil beneath them. Also, occasionally on shaded, mossy banks.

Distribution: In the Olympic Mountains and Cascade Mountains of Washington (U. S. A.), in the Selkirk Mountains of British Columbia (Canada), in the Northern Rockies (Canada), and in northern Alaska.

Specimens seen: Alaska: Pt. Barrow (Lehnert's collection, U. of M.[1]). British Columbia: Glacier—(Fink 4916, U. of M.), beside Great Glacier, 6500 ft. (Fink 5909, U. of M.), Hermit Mt., 6000–7000 ft. (Fink 5768 & 5797, U. of M.); Golden—(Kujala, Herb. Mo. Bot. Gard. No. 69142—type specimen of *C. tenuifolia* var. *pseudoislandica* f. *septentrionalis* Räsänen); Yoho Nat'l Park—Yoho River below Lake Duchesnay (Imshaug 6728). Alberta: Jasper Nat'l Park—ridge opposite Angel Glacier on Mt. Edith Cavell, 6200 ft. (Imshaug 7033 & 7037). Washington: Olympic Mts.—Humes Glacier, 5000 ft. (Frye 14, U. of M.), Hurricane Ridge, 5500 ft. (Smith 2358, U. of M.), Crystal Ridge (Smith 14980, N. of M.), Sol Duc Hot Springs District (Eyerdam 1553, U. of M.); Mt. Rainier Nat'l Park—(Plitt, Aug. 1918, U. of M.), Longmire Springs (Fink 4801, U. of M.), Hidden Lake Trail, 5500–6000 ft. (Imshaug 261, 263 and 267), summit of Yakima Peak, 6200 ft. (Imshaug 484), Kotsuck Creek, 4500 ft. (Imshaug 131), eastern slope of Cowlitz Divide, 3000–4000 ft. (Imshaug 155), Eagle Peak Trail (Imshaug 1567), summit of Chutla Peak, 6000 ft. (Imshaug 1623), Nisqually River Mining Camp, 2900 ft. (Imshaug

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[1] U. of M. = University Herbarium, University of Michigan.

1385), Reflection Lake, 4861 ft. (Imshaug 1074), Lake George Trail, 4000 ft. (Imshaug 1940), Mt. Wow, 6000 ft. (Imshaug 1876—type), Cataract Falls, 4100 ft. (Imshaug 510). Oregon (?):[2] Cascade Mts. (Hall, 1871, Farlow Herb., Harvard Univ.).

2. **Pseudocyphellaria rainierensis** sp. nov. FIG. 2.

Thallus 1–2 dm. latus, fragilis, laciniato-lobatus, pallide virido-cinereus, laciniis (1)3–8 cm. longis (0.7)1.5–3.0 cm. latis, imbricatis, marginibus erosis, coralloideoisidiosis, superne plus minusve foveolatus, glaucus, subtus fere ad apicem dense tomentosis, obscure fuscus, apicibus pallidis vel pallidioribus. Pseudocyphellae densae, irregulariter verruciformes, albae. Algae virides, globosae, diam. vulgo 6.4–10.6 μ. Pycnoconidia bacillaria, recta, 4.2–5.4 × c. 1 μ. Apothecia desunt. Thallus K + intense lutescens, K(C)—, C—, J—, Pd—; medulla K—, K(C)—, C—, J—, Pd—. Specimen typicum in cortice, 2400 ft., Mt. Rainier National Park, Washington, U. S. A., Imshaug 598, in Herb. Mich.

Thallus large, 1–2 dm. across, brittle, laciniate-lobate, pale greenish-cinereous. Laciniae (1)3–8 cm. long, (0.7)1.5–3.0 cm. broad, imbricate; apices broadly rounded, incised crenulate; margins erose to crenate-lacinulate, bearing abundant, coralloid isidia; surface unevenly impressed to foveolate, glaucous, occasionally with isolated clusters of coralloid isidia (not limited to cracks or other injuries, although especially abundant in such areas), endotrophic cephalodia occasionally visible as low warts above surface. Lower side pale buff to brownish yellow towards center, tomentose to the margin; tomentum usually dense, pale. Pseudocyphellae conspicuous, dense, white, 0.2–0.6 mm., verruciform or flattened, margin indistinct. Pycnoconidangia, when present, prominent, superficial, immersed, with the visible part black. Apothecia not seen.

Thallus 0.3–0.4 mm. thick. Upper cortex 38–50 μ, yellowish-brown, paraplectynchymatous; cells (KOH) rather indistinctly arranged in c. 6 horizontal rows, 5.3–7.5 × 3.5–4.3 μ, elongated horizontally, moderately thin-walled. Algal layer 37–45 μ; algae green, 6.4–10.6 μ in diam. Medulla gray, 215–235 μ; hyphae ± intricate, 3.8–4.2 μ, leptodermatous, not granular. Lower cortex yellowish-brown, 32–44 μ, cells similar to those in upper cortex. Rhizinae fasciculate, pale yellow-brown, c. 125 μ long; hyphae 6.4–9.5 μ thick, thin-walled, indistinctly septate. Endotrophic cephalodia with blue-green algae. Pycnoconidangia 0.4 × 0.3 mm.; pycnoconidia bacillar, straight, 4.2–5.4 × c. 1 μ.

Chemical reactions: Thallus K + (intense yellow), K(C)—, C—, J—, Pd—; medulla K—, K(C)—, C—, J—, Pd—.

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[2] The word Oregon on the label probably refers to the Oregon Territory and the specimen could have come from what is now the state of Washington.

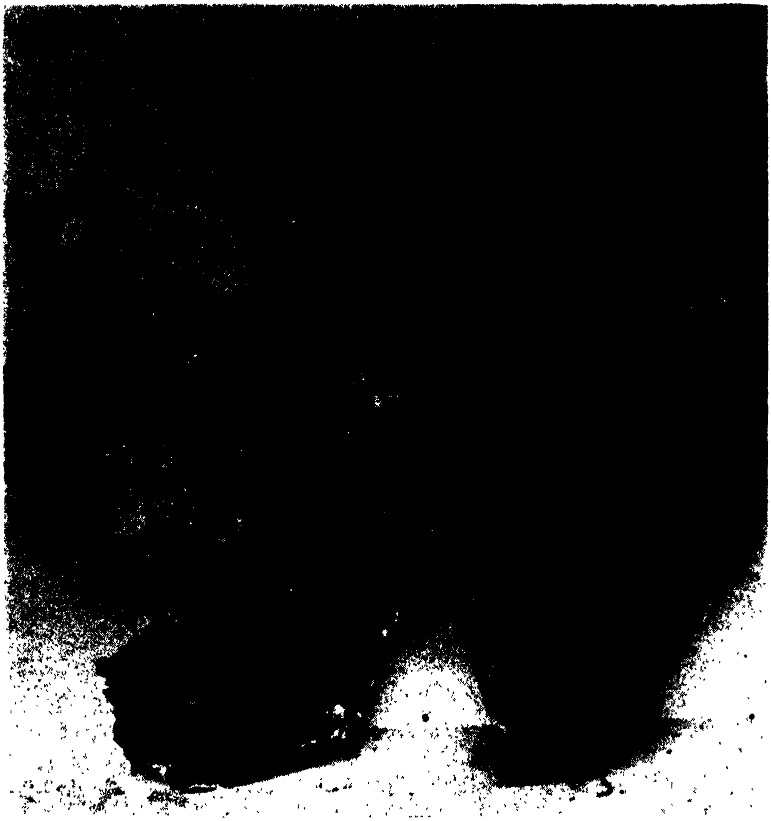

FIG. 2. *Pseudocyphellaria rainierensis.*

Habitat and distribution: On bark of alder and fir at elevations of from 2300–2700 ft., Ohanapecosh River above Panther Creek, Imshaug 598—**type**; Ohanapecosh River above Laughing Water Creek, Imshaug 722; Nisqually River, Imshaug 1362; Tahoma Creek, Imshaug 1238.

Discussion: This species, in many respects, reminds one of *Lobaria oregana,* but differs principally in the presence of pseudocyphellae on the under surface, although the color, texture, and KOH-reaction are also at variance. Points of similarity are the large size, ± foveolate upper surface, extreme brittleness, and coralloid isidia. Also, the endotrophic cephalodia, with blue-green algae, are similar to those described for *L. oregana* by Schneider

(**1897**, p. 58). The large size of *Pseudocyphellaria rainierensis* and the diagnostic characters mentioned above prevent its confusion with any other North American lichen. *Cyanisticta Hookeri* var. *septentrionalis* Räsänen (**1933**, p. 17) appears similar from its description. A study of the type specimen, however, showed that it differs in its much smaller size and in the questionable presence of white pseudocyphellae, as well as in the nature of the algae.

Pseudocyphellaria rainierensis should be looked for in the Olympic Mountains of Washington, U. S. A., and the Selkirk Mountains of British Columbia, Canada. It will most likely be limited in its distribution to these regions of heavy precipitation in our Pacific Northwest.

3. ALECTORIA ALTAICA (Gyel.) Räs., Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo **12** (1): 34. 1939.

A. spinulosa Ahlner, Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo **9** (1): 35. 1937 (nomen nudum).

A. karelica Räs., Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo **12** (1): 34. 1939.

A. altaica var. *spinulosa* Räs., Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo **12** (1): 34. 1939.

Bryopogon altaicus Gyel., Tisia **2**: 166. 1937.

Original description: "Thallus erectus, usque ad 4 cm longus, sat opacus, ad basin nigricans, versus apices pallidus K + flavus, C—, KC + flavus dein celeriter evanescens, P + citrinus deindeque rubescens, in partibus nigrescentibus K—, C—, KC—, ramis divaricatis non rectangulis, rariter subrectangulis instructus, sorediosus, sorediis lateralibus, maculiformibus, sat elongatis vel subrotundatis, albidis, maculis sorediorum vulgo latioribus sicut ramis, ca. 0–1 : 0–0,6 mm magnis. Pseudocyphellae desunt. Sterilis. Species sectionis *Ochroleucae* Gyel.—Similis *Bryop. Berengeriano* (Mass.) Gyel. sed thallus K + et KC +, etc.—U. S. S. R., Sibirien, Altaigebirge, leg. N. N. Lawrow, comm. M. P. Tomin, no. 20 (Typus in hb. mus. Budapest)."

Figures: Acta Phytogeographica Suecica XIII (1940), Pl. 3.

Discussion: This *Alectoria* has previously been reported from North America only from Maine (Degelius, **1940**) and North Carolina[1] (Degelius, **1941**). Outside of North America it appears to be widely distributed in boreal regions. Ahlner (**1940**) reports it from the Ural Mountains, Siberia, Middle Europe and Scandinavia. In view of the fact that the species of the genus *Alectoria* have been badly confused in North America, the original description of *A. altaica,* generally unavailable to American students, has been reprinted. In all probability this species is widely distributed in boreal North America.

A. altaica is well characterized by its erect to short pendulous habit, the dull, bicolored filaments, the soralia, when present, being farinose, the divergent fibrils, and the KOH + (yellow) reaction. *A. nidulifera* (= *A. chalybeiformis* amer. auct., non. *Lichen chalybeiformis* Linn.) is wiry cespitose, lustrous, not bicolored, and the soralia are covered with spine-like isidia. *A. bicolor* is lustrous, bicolored, esorediate and KOH—. *A. jubata* var. *prolixa* is lustrous, pendulous, lacks the divergent fibrils and is KOH—. *A. implexa* is lustrous, pendulous, pale, lacks the divergent fibrils and is KOH + (yellow).

The Mt. Rainier specimen is sorediate and sterile. It was collected on the bark of *Abies* along the Nisqually River, at an elevation of c. 2700 ft., Imshaug 1364.

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A NEW SPECIES OF DERMATOCARPON

HENRY A. IMSHAUG

(WITH 1 FIGURE)

During the summer of 1948, while travelling through Yellowstone National Park the writer collected an interesting *Dermatocarpon*. This lichen formed loose, subspherical mounds on soil in a sage brush desert association near the northeast entrance to the park. The unattached mounds gave the appearance of being able to drift with the wind in dry seasons, thus resembling the familiar tumbleweed. In this habit they remind one of *Parmelia molliuscula* and *Lecanora Haydenii,* both characteristic of arid regions of Western United States. *Cetraria islandica* f. *vagans,* described from the desert regions of Siberia by Savicz (**1911**, p. 51), is also similar in that it forms loose, drifting, spherical mounds.

While examining the collections of *Cornicularia* in the Farlow Herbarium, Harvard University, the author was surprised to find a collection of this *Dermatocarpon* from Mt. Aylmer in the Canadian Rockies. It was distributed by Macoun as No. 105 of Canadian Lichens. Howe (**1915**, p. 225) says of this number: "appears to be abortive or degenerate material" of *Cetraria Richardsonii.* Although Howe locates Mt. Aylmer in British Columbia, the only Mt. Aylmer the writer is able to locate is in Alberta, on the eastern boundary of Banff National Park, just north of Lake Minnewanka.

An epithet indicative of its drifting habit is deemed suitable for this new species.

Dermatocarpon vagans sp. nov. FIG. 1

Thallus foliaceo-fruticosus, iteratim laciniatus, laciniis primariis 3–6 mm. latis, convolutis, apicibus tenuissimis, irregulariter ramosis et crispatulis. superne plus minusve glaber, pruinosus, siccus spadiceus, madefactus virescens; subtus obscurus vel ater, papillosus. Perithecia immersa, ostiolis nigris. Sporae ellipsoideae, 11.0–15.4 × 6.2–7.5 µ. Thallus et medulla K—, K(C)—, C—, J—, Pd—; gelat. hym. J + obscure caerulescit. Specimen typicum in solo, Yellowstone National Park, Wyoming, U. S. A., Imshaug 15, in Herb. Mich.

Thallus large, 2–4 cm. across, foliose-fruticose, forming subspherical, unattached mounds on arid soil. Thallus irregularly and deeply laciniate, not in one plane, laciniae projecting at various angles; ends of main laciniae repeatedly dissected and the many lacinulae strike out at all angles, resulting in small tight balls at ends of main branches. Laciniae convolute, main divisions 3–6 mm. across (dry), apices c. 0.5 mm. Upper surface glabrous to minutely rugose; scattered areas white-pruinose; when dry date-brown, green when moistened. Lower surface, rarely visible due to convolute nature of the laciniae, brownish-black, densely papillose. Rhizoids none. Soredia and isidia none. Perithecia numerous, immersed, ostiole black, minute. Pycnoconidangia not observed.

FIG. 1. *Dermatocarpon vagans.*

Thallus 0.4–0.6 mm. thick. Upper cortex 23.5–30.0 μ, plectynparenchymatic, colorless to pale fuscous above, cells isodiametric, thin-walled, 5.3–8.5 μ, formed from vertical hyphae. Algal layer 80–120 μ thick; algae pleurococcaceae, globose, diam. 7.5–10.5 μ. Medulla lax, 200–325 μ thick, hyphae intricate, thin-walled, 2.2–2.6 μ. Lower cortex 40–85 μ thick, plectynparenchymatic, cells isodiametric, thin-walled, 6.4–10.6 μ, formed from vertical hyphae, interior cells colorless, outer ones (forming papillae) brown. Perithecia immersed, ± globose, pallid to pale rose, diam. 250–300 μ, wall 40–80 μ thick, formed from longitudinal hyphae, margin of the ostiole obscure. Paraphyses indistinct, soon gelatinized. Asci subcylindric, 54–60 × 13–15 μ. Spores 8, in two rows, hyaline, ellipsoid, granular, non-septate, 11.0–15.4 × 6.2–7.5 μ (length/breadth-coefficient = 1.7–2.3 μ, average 2.0 μ), membrane thin.

Chemical reactions: Thallus and medulla K—, K(C)—, C—, J—, Pd—; gelat. hym. J + (blue).

Habitat and distribution: Growing loose on arid soil, c. 7000 ft., in northeast corner of Yellowstone National Park, Wyoming, June 23, 1948, Imshaug 15—**type**; growing over moss, 8000 ft., Mount Aylmer, Alberta, Canada, Macoun, Canadian Lichens No. 105 (distributed as *Cetraria odontella* (?) Ach.).

Discussion: *Dermatocarpon vagans* is near *D. miniatum* and should be compared with it and the other large-lobed species in the section Entosthelia. *D. miniatum* itself is a widespread and variable plant, characterized by a generally pruinose upper surface, lower surface without rhizoids, and the color unchanging when moistened. It may be monophyllous or polyphyllous, varying from a somewhat imbricate condition to a few medium-sized lobes (var. *complicatum*) or to a compact cushion of many small, densely-congested lobes (var. *panniforme*). These polyphyllous forms are often mistaken for *D. aquaticum.* The lower surface of *D. miniatum* varies in color from yellow-fulvous to fuscescent or black. In texture, the lower surface varies from smooth or rugose to reticulate-veined (var. *fulvofuscum*) or may be papillose (var. *papillosum*). It is the papillose forms which present the greatest difficulties. Although not recognized by Fink (**1935**), *D. miniatum* var. *papillosum* has been reported from Washington by Magnusson (**1932**) and from New Mexico by Bouly de Lesdain (**1942**). *D. moulinsii* var. *subpapillosum* Fink apud Hedrick (**1933**, p. 306) would appear from a study of the type specimen, which is rather meager, to be this variety. *D. vagans,* aside from its habitat and gross appearance, differs from *D. miniatum* in that the color changes to green when moistened.

D. reticulatum Magnusson (**1932**, p. 18), described from the Upper Naches River Region in the Cascade Mts. of Washington, is still incompletely known. It differs from *D. miniatum* var. *papillosum* in its reticulate under surface and in the change of color to green when moistened. Also, the under surface in *D. reticulatum* seems to be more furfuraceous or granulose than papillose. Specimens of a collection from Tipsoo Lake, Mt. Rainier National Park, near the type locality, made by the writer (Imshaug 220) and determined by Magnusson, show considerable variability in the prominence of the rugae. In some they may even be absent. While the thallus of the type specimen was described as 150–250 μ in thickness, thinner than in *D. miniatum,* the writer's specimens are 400–600 μ in thickness. More material is needed of this and the other papillose forms to ascertain the true relationships. *D. vagans* differs from *D. reticulatum* in the lack of prominent rugae and in the more strongly papillose lower surface, in addition to habitat and gross appearance.

The following key to the large-lobed Dermatocarpons of the section Entosthelia summarizes these differences. The distributions given have been compiled from North American material in the University Herbarium, University of Michigan.

A. Lower surface smooth or wrinkled.
  B. Thallus green when moistened, not pruinose; generally on wet or inundated rocks.................................*D. aquaticum*
    (New England, Que., N. Y., Ont., Mich., Wisc., Ohio, Md., D. C., Ark., Colo., and Wash.)
  BB. Thallus unchanged when moistened, pruinose; on exposed rocks. *D. miniatum*
    (throughout the United States and Canada)
AA. (see AAA). Lower surface furfuraceous or papillose.
  C. On rock; thallus ± plane, not laciniate.
    D. Unchanged when moistened; lower surface without prominent rugae.........*D. miniatum* var. *papillosum*
      (Wash., Ore., Idaho, B. C., Manitoba, Mont., and Colo.)
    DD. Olive-green when moistened; lower surface with prominent rugae..........................*D. reticulatum*
      (Washington)
  CC. On soil, loose; thallus foliose-fruticose, deeply laciniate, convolute......................................*D. vagans*
    (Wyoming and Alberta)
AAA. Lower surface with rhizoids...........................*D. moulinsii*
  (Mich., Ont., Colo., Mont., and Wash.)

It may be noted here that the Washington specimens of *D. moulinsii* (Imshaug 178 and 934B) are from Mt. Rainier National Park and are larger than usual for the species (thallus 2.5–7.0 cm.

across, 250–400 μ thick) and with long (1.5–3.0 mm.), branched, pale yellow-brown to black rhizoids. Also, the lower surface has plates of supporting tissue similar to that in *Umbilicaria Muhlenbergii.* Duplicate material of No. 178, however, was determined as *D. moulinsii* by Magnusson. The large size and the branched rhizoids suggested *D. vellereum* Zschacke (**1934**, p. 638) but the rhizoids, although branched, are not densely coralloid, as in Elenkin's Lichenes Florae Rossiae No. 49a (Univ. of Mich. Herb.).

I wish to take this opportunity to express my appreciation to Dr. A. H. Magnusson, Göteborg, Sweden, for the determination of specimens and to Dr. W. L. White for the use of the facilities of the Farlow Herbarium, Harvard University.

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SOME LEAFSPOT FUNGI ON WESTERN GRAMINEAE. V[1]

RODERICK SPRAGUE[2]

(WITH 1 FIGURE)

This paper continues a series of discussions and descriptions of fungi found on the grass family in the far west (**10**).

VISCOMACULA gen. nov.

Hyphis in maculis in foliis vivis, congregatis in tenues pulvinos, sporulis massas lentas habentibus, hyalinis, brevibus cylindraceis.

Hyphae in spots on living leaves, grouped in extensive shallow pads, spores formed in sticky masses, hyaline, short cylindric. Differs from *Rhynchosporium* in the appreciably thicker mycelial pad and in the gelatinous spore masses.

Viscomacula aenea sp. nov.

Macula aenea, margine aureo, centro pallido-aeneo, 4–20 × 2–4 mm.; hyphis hyalinis et in foliis et pulvinis tenuibus superficialibus 2–6 cellas crassis; sporulis tenues aeneas lentas massas, ultimo aridas habentibus, hyalinis, brevibus cylindraceis, apice rotundato, raro basi subacuminata, aseptatis, pseudo-multiseptatis (duas vel plures guttulas habentibus), (5.6)6–9(10) × (1.4)1.6–2.0(2.3) μ.

Hab. in foliis vivis *Poae amplae* Merr., inter Loveland Pass et Silver Plume, Colo.; Amer. Bor. Typus est A.S. 20,538. Legit. Sprague, R., Fischer, G. W. et Meiners, Jack P. Aug. 8, 1948.

Spots copper colored, margins yellow, center pale copper colored, 4–20 × 2–4 mm., hyphae hyaline in the foliage and also aggregated on the leaf surface in interrupted shallow gelatinous masses 2–6 cells thick. Spores in small, sticky, copper or carrot-colored masses, finally hard and dry, and then obscure; spores borne on ill-formed conidiophores on the flat fruiting areas, short cylindric, ends rounded or sometimes one end somewhat pointed, hyaline or very faintly yellowed, contents biguttulate and giving appearance of a wide central septum or rarely 2 such cleared spaces, but no true cross walls seen, (5.6)6–9(10) × (1.4)1.6–2.0(2.3) μ.

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[1] Published as Scientific Paper No. 905, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington.

[2] Pathologist, Tree Fruit Experiment Station, Wenatchee, Washington.

Habitat in living leaves of *Poa ampla* along a stream-side meadow below Loveland Pass and about 10 miles up U. S. Highway 6 from Silver Plume, Colo. Type is A. S. 20,538, consisting of about 12 leaves of which only 4 or 5 are good material.

This fungus, collected at an elevation of about 9500 feet, is an active parasite, causing well-defined spots on leaves in part of the somewhat scanty type. It is distinct from anything else which we have seen. The fruiting pad is too well-developed for *Rhynchosporium orthosporum* Caldwell and its apparent sticky or somewhat gelatinous spore mass distinguishes it from the dry spore masses of *Rhynchosporium.* It reminds one of copper spot, *Gloeocercospora sorghi* D. Bain and Edg., but the spores in the Colorado fungus are utterly different. They also differ radically from *Ramulispora.* Its definite moniliaceous nature tends to exclude it from *Gloeosporium. G. meinersii* f. *alpina* on *Poa alpina* Merr. has larger spores, 10–14 × 3–4 μ (10).

The fungus is apparently an uncommon genus on alpine or subalpine grasses. The region, about 10 miles above Silver Plume, Colo., along U. S. Highway 6, was a favorable area for collecting sub-alpine leaf spots.

MATERIAL on *Deschampsia atropurpurea* collected by Fischer at Logan Pass, Mont., was partly destroyed by insects in the packet before examination was completed. Spores were still present, but no fruiting bodies remained. The spores were of one species. They may represent a fungus of mycological interest because the insect-riddled lesions indicated that the remaining fungus may have originally caused the spot. The spores are pale brown, elliptical-cylindrical, 8.5–14 × 4.2–5.7 μ (FIG. 1, *A*). Further material is needed to determine whether this fungus is only a saprophyte on the insect frass or is a parasitic fungus. If pycnidia were present (*Sphaeropsis*), they certainly were completely destroyed. If it is a member of the micronemae of the Dematiaceae of the Moniliales, a new genus would need recognition.

Stagonospora spartinicola sp. nov.

Maculis incoloratis v. stramineis, elongatis; pycnidiis obscuris, immersis, sub-globosis, parenchymatis, brunneis, ostiolatis, 150–280 μ diam.; pycno-

sporulis cylindraceis; constrictis ad septa, utrinque rotundatis, contextu guttulatis, hyalinis, 3-5 septatis, 40–47 × 6–9 μ.

Hab. in foliis vivis et emortuis *Spartinae pectinatae* Lk., prope Wilton, N. Dak., Amer. Bor. B.P.I. 80,798, Sept. 14, 1941 (type) et Madison, Wisc. (H. C. Greene) Aug. 13, 1949.

Spots none or when present straw color, drab, faintly buff or light tan, elongate up to 60 × 1.5 mm. diam.; pycnidia immersed but with a relatively large ostiole visible through the slight rupture in the host epidermis, sub-globose to moderately flattened or elliptical in outline, thin walled, parenchymatous, pale brown becoming nearly hyaline in the vicinity of the ostiole, 150–280 μ diam.; pycnospores cylindrical, constricted at the septa, ends rounded but one more regularly so than the other, contents coarse including a large chlorinous guttula, 3–5 septate, 40–47 × 6–9 μ.

On living or dead leaves of *Spartina pectinata* near Wilton, N. Dak. and Madison, Wisc.

This fungus is characterized by the sunken, flattened, pale-colored pycnidia and the cylindrical spores (FIG. 1*B*) which are larger than those of *St. vexatula* Sacc. to which they show closest relationship. *St. vexatula* on *Phragmites* has spores 35–38 × 4.5–5.0 μ, but some are as long as 55 μ. *Septoria arundinacea* Sacc. has spores 60–70 × 5–6 μ. They are longer and narrower than the fungus on *Spartina.* We have been reluctant to consider this fungus as undescribed because of the complex of related forms on coarse marsh grasses. These pycnidial forms involve a consideration of not only *St. vexatula* but nearly twenty other species descriptions. A detailed study of this group indicates, however, that *St. spartinicola* has wider, coarser spores than any of them. The type material from North Dakota, which was apparently saprophytic, is supplemented by a fragment sent by H. C. Greene from Madison, Wisc. The lesions in this material are well delineated and as much as 60 × 1.5 mm. diam. The spores in each collection are very similar, both having large chlorinous guttulae and both varying in number of septa from three to five. The species is likely to prove to be one having seven septa in overwintering material. The spores are cylindrical, but one end tends to be slightly blunter than the other. This aspect, plus strong but variable constrictions, gives the spores a rugged, coarse appearance which I have not been able to completely portray in a simple line drawing.

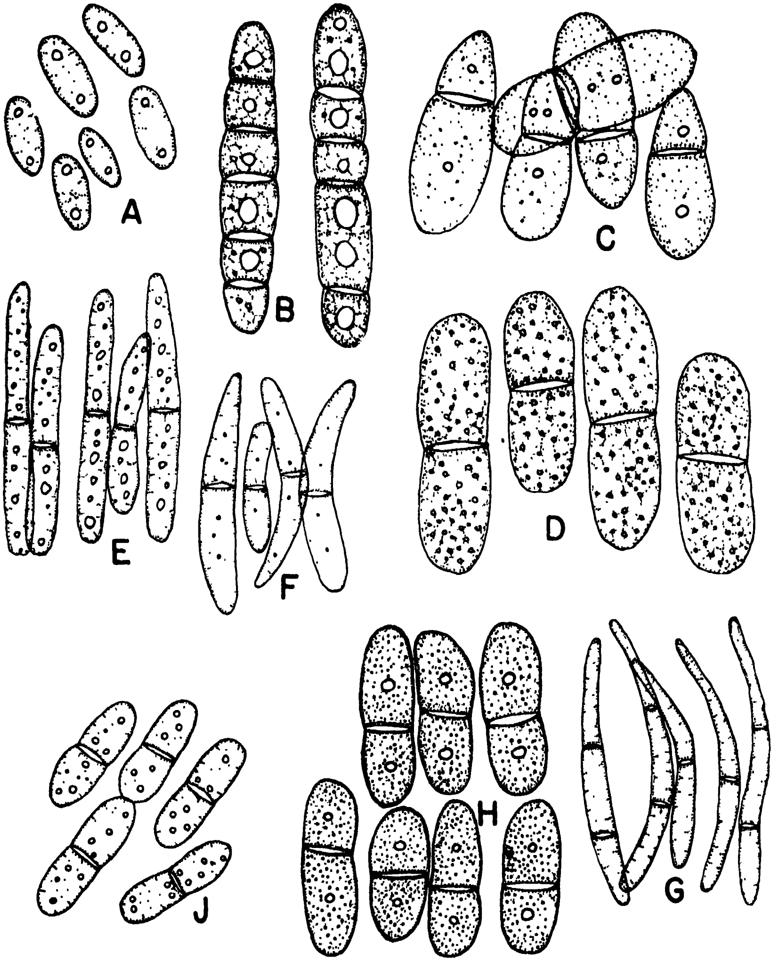

FIG. 1. Spores drawn with the aid of the camera lucida, × 1,000. *A*, Undeterminable, from insect-eaten spots on *Deschampsia atropurpurea*. *B*, *Stagonospora spartinicola* on *Spartina pectinata*, type. *C*, *Apiocarpella macrospora* on *Poa ampla*, near Silver Plume, Colo. *D*, *Stagonospora simplicior* on *Melica bulbosa*, Waterton Lakes Nat'l Park, Alta. *E*, *Septoria avenae* on *Calamagrostis canadensis*, Baker Hole, Mont. *F*, *Scaphidium boutelouae* on *Bouteloua curtipendula*, Santa Catalina Mts., Ariz. *G*, *Phleospora muhlenbergiae*, from type. *H*, *Stagonospora simplicior* (*Ascochyta* summer stage) on *Stipa columbiana* var. *nelsonii*, Mt. Shasta, Calif. *J*, *Ascochyta boutelouae* (cfr. *Diplodina graminea*) on *Sporobolus airoides*, Pullman, Wash.

ADDITIONAL SPECIES

APIOCARPELLA MACROSPORA (Speg.) Sydow was found on a few leaves of *Poa ampla* Merr. below Loveland Pass on U. S. Highway 6 toward Silver Plume, Colo., by the writer, Fischer, and Meiners (A. S. 20,537). One or two dark brown pycnidia with closed (but developing) ostiolar regions occurred in fawn-colored, elliptical spots on living leaves. The spores (FIG. 1*C*) differed from saprophytic material of *A. macrospora* on *Stiporyzopsis* from California (9) in that most, but not all, of them were broadly cylindrical and blunt, even at the smaller end. They measured wider (8.5–10 μ, compared with 6.6–8.8 μ) but not longer (21.4–26 μ, compared with 21–26 μ) than the California collection. Since some of the Colorado spores were pointed at the smaller end and typical of *A. macrospora,* it is questioned if this collection warrants separating from the California specimen previously discussed.

STAGONOSPORA SIMPLICIOR Sacc. and Berl. is not uncommon as a parasite of *Stipa* spp. and other grasses in western mountains. The fungus on *Stipa* usually has 1-septate, short, thick, spores (FIG. 1*D*) which we believed might be *Ascochyta stipae* Died. On comparing further, it became evident that Diedicke (2) illustrated a different fungus. His fungus had thick-wall spores, whereas, *St. simplicior* has thin-wall spores and a strong constriction at the central septum.

The material on *Stipa* was arranged for separation from the species proper and a description prepared. Further study showed that the parasitic summer material developed shorter spores (FIG. 1*D*), but all gradations were found. It is apparent that *St. simplicior, St. simplicior* var. *andropogonis* Sacc. and our material on *Stipa* and other mountain grasses can just as well be grouped together. It will be well, however, to publish an English description of the summer developing phase on *Stipa,* since it differs somewhat from the original description of the species:

Spots maroon to vinaceous, sub-circular, prominent but scattered on the leaves, centers of spots paler, finally straw color; pycnidia few, large, globose, erumpent, golden-brown, 150–200 μ diam.; spores broadly cylindrical, ends bluntly rounded, strongly constricted at the central septum, sometimes each half again partially divided by developing septum, guttulate, 1-septate phase 17.5–26 × 6.5–9.5 μ, 1- to 3-septate phases occur on dying leaves, 24–31 × 9–11.0 μ, hyaline. Differs from winter material and from var. *andropogonis* in having less robust spores.

Hab. in living or necrotic leaves of: *Stipa columbiana* Macoun, Medicine Bow Nat'l For., Wyo. (A. S. 20,153); *S. columbiana* var. *nelsonii* (Scribn.) Hitchc., Mt. Shasta, Calif., Utah; *S. lemmonii* (Vasey) Scribn., Malheur Nat'l For., Oregon; *S. lettermanii* Vasey, Skyway, Colo.; *S. occidentalis* Thurb., 10 miles n.w. of Sisters, Oregon (A. S. 20,511); *S. viridula* Trin., North Dakota; *S. williamsii* Scribn., Medicine Bow Nat'l For., Wyo. (A. S. 20,526); *Stiporyzopsis bloomeri* (Boland) Johnson, Mt. Whitney, Calif.

While the material on *Stipa,* which was found on living leaves, is typically that of a large-spored species of *Ascochyta,* we have found spores in pycnidia on dead leaves of *Stipa* that are longer, broader, and with 3 septa forming. These spores are very similar to immature ones of *Stagonospora simplicior. St. simplicior* is a common saprophyte on a number of grasses (**10**) but is not infrequently now found to attack healthy plants. Greene has collected material of *St. simplicior* f. *andropogonis,* on several hosts from Wisconsin, that appeared to be actively parasitic. The living leaves harboring the *Ascochyta* phase of the *Stipa* collections have very evidently been invaded by the fungus. Saprophytic material, however, occurs on darker dead leaves in *Stipa columbiana* and *S. lettermanni.* The pycnidia on *S. lettermanii* contain spores 24–30 × 9.0–10.8 μ. Those on *S. columbiana* have very similar spores 24–31 × 9–11 μ.

Stagonospora simplicior on *Melica bulbosa* (FIG. 1*D*) was growing with *Septoria nodorum* Berk. in tawny spots on living leaves and sheaths collected by G. W. Fischer at Waterton Lakes Nat'l Park, Alta., Aug. 7, 1949 (A. S. 20,504). While *S. nodorum* was more abundant in the lesions than the *Stagonospora,* both were apparently parasitic. The spores of the *Stagonospora* were coarsely cylindrical, strongly constricted at the first, later at the third septum, somewhat blunted at the ends, the contents coarsely granular but hyaline, 26.8–36 × 9.8–12.8 μ (FIG. 1*D*). The pycnidia were much larger than those of *S. nodorum* (about 150–180 μ as contrasted with

90–140 μ), but otherwise they were similar in appearance in the spots. The *Stagonospora* spores, however, did not readily ooze from the pycnidia as did the much smaller and maturer *Septoria* spores.

We also have some material on *Festuca ovina* L. (A. S. 20,527) from Skyway, Colo., with yellow spores 29–38.6 × 8.5–12.1 μ borne on dead brown leaves on living plants. This fungus also is *St. simplicior* and similar to another collection on *F. ovina* var. *brachyphylla* from Skyway (**10**). A collection on *Sporobolus airoides* from Pullman, Wash. (FIG. 1*J*), was withheld from a monographic discussion of *Ascochyta* by myself and A. G. Johnson (**11**) because the spores had the same general shape as those of *St. simplicior* although they were very much smaller. It probably is a species of *Ascochyta* and resembles *A. boutelouae* Fairman which is the same as *Diplodina graminea.* A combination placing the last mentioned in *Ascochyta* was made elsewhere (**11**).

Ascochyta stipae Gonzalez-Fragoso (**3**) has ovoid-oblong spores 7–8 × 2.0–3.5 μ. It is, therefore, entirely distinct from *St. simplicior. Ascochytella stipina* Petrak (**4**) also has much smaller spores than *St. simplicior,* 7–13 × 3–5 μ.

SEPTORIA TRITICI Rob. in Desm. was found on dead leaves and sheaths of rye plants (*Secale cereale* L.) collected by B. F. Dana in acid soil at Shelton, Wash., June 23, 1919 (C. S. 679). The plants were also affected with *Ophiobolus graminis* Sacc. and *Rhizoctonia solani* Kuehn, both attacking the base of the culm (**1**). The *Septoria* has typical microspores and straight or curved 3-septate macrospores 45–70 × 1.4–1.7 μ. The fungus was unquestionably *S. tritici* even though the host is certainly unusual. We have no record of this species on rye in this country. The host was growing in a humid region in acid, infertile soil and was attacked by rootrot and footrot organisms. This probably accounts for the presence of this fungus on the necrotic rye. *S. secalis* and *S. nodorum* have much wider-appearing spores than *S. tritici.*

SEPTORIA AVENAE Frank caused a tawny spot on the leaves of *Calamagrostis canadensis* L. at Baker Hole near West Yellowstone, Mont., collected Aug. 13, 1948 (A. S. 20,107), by Fischer, Meiners, and myself. The hyaline spores with somewhat granular contents were tapered towards the apex and definitely blunted at the base (FIG. 1*E*). The spores were only 1-septate, 21–28 × 3.8–4.8 µ. They are, therefore, shorter and broader than *S. avenae* on oats. The pycnidia are comparable, however, light golden brown, 90–130 µ diam., borne in typical lesions. This fungus could, of course, be classed as a 1-septate phase of *Stagonospora arenaria* Sacc., but this fungus appears to be less frequently encountered on the Agrostideae than on the Hordeae of the Gramineae. *S. avenae* was seen on *Agrostis exarata* Trin. from northern Idaho.

SEPTORIA ARUNDINACEA Sacc. was found on dead leaves of *Deschampsia caespitosa* (L.) Beauv. on the shore of Priest Lake, Ida., by the writer and Mary Sprague (A. S. 20,542). The spores in this material are very large, 60–92 × 4.8–6.0 µ, slightly tinted, with small oil drops. They were bacillar, somewhat squared at the ends, slightly to scarcely constricted at the 3–6 septa, and somewhat brittle. The pycnidia were large, sometimes multilocular with 1 to 3 ostioles, strongly erumpent. This fungus is closer to *Septoria* than to either *Phaeoseptoria* or *Hendersonia*. This material is the first which I have seen from western America, although it probably is common on coarse marsh grasses in the eastern United States.

I doubt if the fungus is actively parasitic on *Deschampsia caespitosa*, a host resistant to most fungi.

ASCOCHYTA BRACHYPODII (Sydow) Sprague and A. G. Johnson (11) was found on *Andropogon scoparius* Michx. near Flagstaff, Ariz., by Sprague, Fischer, and Meiners (A. S. 20,543), on overwintered leaves. The spores had pointed ends as in North Dakota material on *Andropogon*. The collection represents an extension of range and host for the fungus.

SCAPHIDIUM BOUTELOUAE Clements was collected on *Bouteloua curtipendula* (Michx.) Torr. by W. G. Solheim and Ragnhild Solheim from the Santa Catalina Mts., Ariz. (Rocky Mtn. Herb. 2447) and Silver City, N. Mex. (Rocky Mtn. Herb. 2390). The fungus produces linear brown or gray lesions with brown borders, or in later saprophytic activity the entire lesion may be gray or dull black and with indefinite borders. In collection 2447, the brown-bordered lesion is surrounded by green tissue and is evidently the result of parasitic invasion of the living leaf. These lesions were 1–4 × 0.4–1 mm. diam. with fawn-colored centers.

The pycnidia were black, flattened globose to somewhat elongated, breaking open at the apex and 60–100 μ diam. The spores were curved, fusiform-obclavate (FIG. 1*F*), yellow, 19–30 × 2.8–5.3 μ. The type of this is not available for study, and I based my determination on the description.

PHLEOSPORA MUHLENBERGIAE Sprague and Solheim on *Muhlenbergia arizonica* Vasey was recently described (5). An illustration of the pycnospores is included at this time (FIG. 1*G*).

PHYLLOSTICTA OWENSII Sprague was originally found on *Dactylis glomerata* L. along the seacoast in Oregon. A. C. Goheen collected comparable material in a bog near Long Beach, Wash., July 19, 1949 (A. S. 20,510), on a very different host, *Panicum capillare* var. *occidentale* Rydb. The obscure lesions on the basal and sub-basal leaves are gray to brown. The pycnidia are small and black, containing bacillar-shaped spores, 3–4 × 1.0–1.4 μ, which are exuded in agglutinated masses. This material is distinct from *P. panici* E. Young and *P. sorghina* Sacc.

SPERMOSPORA SUBULATA (Sprague) Sprague is associated with a light buff, elliptical red-bordered spot on *Trisetum spicatum* (L.) Richt., collected by G. W. Fischer in high country at Logan Pass, Glacier Nat'l Park, Mont. (A. S. 20,501). This is a new host-genus for the fungus. Fischer also collected the same fungus on *Bromus vulgaris* (Hook.) Shear in a devil's club thicket at Avalanche Lake in Glacier Nat'l Park, Mont. (A. S. 20,503). The lesions were gray, blasted areas on living leaves. A fungus which occurs on *Stipa lettermanii* Vasey near Cameron Lake, Waterton Nat'l Park, Alta., is also assigned to *S. subulata* (A. S. 20,506, legit. G. W. Fischer, 1949).

CERCOSPORA BOUTELOUAE Chupp and Greene was collected by the writer and Jack Meiners along Buckhorn Creek, Roosevelt Nat'l For., Colo., in Aug. 1948, on *Bouteloua gracilis* (H. B. K.) Lag., a new host. The spores were light brown, otherwise the same as the fungus described by Chupp and Greene. The narrow spots on living leaves have ashy centers, while those on dead leaves are usually uniformly dark because of the accumulation of conidiophores. Part of this material was sent to Greene who forwarded it to Chupp for study. *C. bouteloua*e is related to *C. apii*.

OVULARIA PUSILLA (Ung.) Sacc. and D. Sacc. causes a dark brown to sepia, elliptical spot on living leaves of *Alopecurus alpinus* J. E. Smith, near Hotel Many Glaciers, Glacier Nat'l Park, Mont. The material was collected Aug. 8, 1949, by G. W. Fischer (A. S. 20,505). Except for a collection of *Puccinia poae-sudeticae* (West.) Jorstd. from Colorado, this appears to be the only parasitic fungus reported on this unusual appearing alpine grass. *O. pusilla* was also collected on *Agrostis oregonensis* Vasey in Glacier Nat'l Park by Fischer. He also brought material of *Elymus glaucus* Buckl. from Kootenay Park, B. C., infected with *O. pusilla*. This seems to be the first report of the fungus from western Canada and the first report of it on the genus *Elymus*. The spots were elliptical in shape and fawn-colored.

Ovularia pusilla also causes a tan leaf blight on the filiform leaves of *Agrostis humilis* Vasey at Togwotee Pass in the Teton Nat'l For., Wyo. (Sprague, Fischer, and Meiners). This appears to be the first report of a parasitic fungus on this tufted alpine bent. *O. pusilla* is much more common on western grasses than is generally believed. The spores are so few in most water mounts that the fungus is frequently overlooked unless one is familiar with the symptoms or notes the small glistening tufts of conidiophores under a binocular (× 45).

RHYNCHOSPORIUM ORTHOSPORUM Caldwell was collected on *Agropyron subsecundum* by Alvin Law and Verne Comstock in Bountiful Canyon, Utah, July 13, 1949. The sub-hyaline spores were extremely variable in size and shape. A few had a faint tendency towards formation of obliquely hooked apices as in *R. secalis*. Some of the spores were straight and typical for *R. orthosporum*, averaging 13–17 × 3–4 μ. Other spores were capsular shaped and so aberrant as to have little resemblance to the others. Some were distinctly swollen and others Indian-club-shaped. The capsular forms averaged about 7 × 4.2 μ. These spores were borne in straw colored, elongate sheath spots with reddish brown borders. They resemble sharp eyespot (*Rhizoctonia solani* Kuehn) or strawbreaker eyespot (*Cercosporella herpotrichoides* Fron) on wheat.

It is likely that the above-mentioned *Rhynchosporium* is not an undescribed species but an aberrant form of *R. orthosporum* modified by heat, following conditions that had been favorable to the fungus. We have seen unusual material of *Rhynchosporium* on *Elymus glaucus* Buckl., in Linn County, Oregon. This fungus, however, develops year after year in late winter in the same restricted area near Corvallis, Oregon. *R. orthosporum* has been collected on *Agropyron subsecundum* near Trude, Ida., but this material is not polymorphic as is the Utah collection. Typical material of *R. orthosporum* was also found on *A. subsecundum* var. *andinum* (Scribn. and Sm.) Hitchc. at an elevation of 11,316 feet on Fremont Pass, Colo. (A. S. 20,532). *R. secalis* was found on *A. subsecundum* at Pullman, Wash.

RHYNCHOSPORIUM SECALIS (Oud.) J. J. Davis was collected on *Phalaris arundinacea* L. at Waterton Lakes, Alta., by G. W. Fischer. The spores are definitely intermediate between *R. orthosporum* and *R. secalis,* but it was assigned to the latter because the spores, or at least some of them, tended to be obliquely hooked at the apex. *R. secalis* was also collected on *Phalaris* near Leavenworth, Wash.

STAGONOSPORA SUBSERIATA (Desm.) Sacc. occurs in brown blotches on leaves of *Elymus mollis* Trin., collected by Austin Goheen on Neptune Beach (Puget Sound), Whatcom Co., Wash. (A. S. 20,539). This fungus was collected on the same host in Oregon (6) with spores 20–33 × 4.5–5 μ. The material from Washington is worthy of note because it gives indications of parasitic activity. Our collections have pycnospores which are narrow for this species, but we believe that they represent the same organism. Goheen's specimen had sunken light brown pycnidia with the apex of each pycnidium protruding, ostiolate, spores yellow, guttulate, fusiform-cylindric, sometimes constricted at the septa, 23–30 × 4–5 μ.

SEPTORIA NODORUM Berk. caused a prominent purplish brown glume blotch on heads of *Agropyron spicatum* (Pursh) Scribn. and Sm. growing in open woods north of Underwood, Wash. (legit. G. W. Fischer and Jack P. Meiners). The spores were 1-septate, 15–18 × 3.0–3.2 μ. This is typical for the summer *Ascochyta* phase of *S. nodorum.* This is apparently the first report on *A. spicatum.* We also have material of *S. nodorum* on *Melica bulbosa* from Skyway, Colo. (A. S. 20,534). This collection has circular to elongate, buff or fawn colored, sometimes vague spots, surrounded by brown tissue. The golden brown pycnidia have cylindrical hyaline or faintly greenish 1-septate spores, 17–20 × 2.4–3.4 μ. This appears to be the same species as W. B. Cooke collected on *Melica californica* Scribn. on Mt. Shasta, Calif. (**9**). *S. nodorum* was collected on *Stipa viridula* Trin. at Gunnison, Colo. The spores were 14–23 × 2.2–2.8 μ, mostly *Ascochyta*-like but somewhat more mature and 3-septate. It is difficult to segregate *S. nodorum* from immature material of *S. avenae.* On the basis of spore size, we must call these *S. nodorum.*

SCOLECOTRICHUM GRAMINIS Fckl. on *Glyceria leptostachya* Buckl., collected near Rochester, Wash. (A. S. 21,622), represents the first report of a parasitic fungus on this comparatively rare grass.

HELMINTHOSPORIUM DICTYOIDES Drechsl. was collected on *Festuca pacifica* Piper near Colfax, Wash. (A. S. 20,518). *H. dictyoides* is not common in the west, even on *F. elatior.* The collection on *F. pacifica* represents the only known specimen of *H. dictyoides* on an annual species of fescue.

STAGONOSPORA AGROSTIDIS f. ANGUSTA Sprague was found mingled with rust pustules of *Puccinia scaber* (Ell. and Ev.) Barth. on leaves of *Stipa robusta* Scribn. near Stout, Colo. (A. S. 20,521). The spores were the same as in the type originally described on *Stipa viridula.*

SEPTORIA GLYCERICOLA Sprague was found in faded spots on *Glyceria grandis* S. Wats. near Lolo, Mont. (A. S. 20,524). The pycnidia were black, thick-walled, small (60–100 μ). The spores were also small, 15–24 × 1.6–1.9 μ, 0–1-septate, filiform clavulate. This material compares with a collection on *G. grandis* from Bismarck, N. Dak. (**8**), which had summer spores, 10–15 × 1.3–1.6 μ. These are believed to be summer phases of *S. glycericola.*

SEPTORIA ANDROPOGONIS J. J. Davis occurs on *Stipa robusta* Scribn. near Stout, Colo. (A. S. 20,528). The spores appear immature, 1-septate, blunt at the base, pointed at the apex, 28–40 × 2.6–3.5 μ. We have found *S. andropogonis* f. *sporobolicola,* with spores 40–73 × 2.4–3.4 μ, on *Stipa comata* Trin. and Rupr. at Mandan, N. Dak. (**7**). We have since found the same fungus on *Stipa viridula* in several states. The material on *Stipa robusta* has shorter, proportionately stouter spores than *f. sporobolicola* but otherwise comparable. Some of the spores are ill-formed. Some have one cell empty, or the spores are distorted or twisted at the apex. The pycnidia are deep seated and occur in dull gray-brown lesions. The specimen on *S. robusta* possesses spores which are somewhat intermediate in size between *S. andropogonis* and *f. sporobolicola.* The entire series of collections available on coarse prairie grasses now indicates that *S. andropogonis* and its subdivisions are not segregated by sharp boundaries. Of course, the extremes, the extremely short *S. andropogonis* and the extremely long var. *sorghastri* Greene and Sprague, are readily distinguishable.

HELMINTHOSPORIUM TRITICI-REPENTIS Died. caused scattered small purple black spots on the leaves of a creeping form of *Agropyron spicatum* (Pursh) Scribn. and Sm. near Worley, Ida. (A. S. 20,544).

HELMINTHOSPORIUM STENACRUM Dreschl. was found on *Agrostis interrupta* L. near Ford, Idaho (A. S. 20,545). The dried brown basal leaves were covered with the fungus. The spores were 65–105 × 14–20 μ diam. borne on simple conidiophores, 60–135 × 6.5–7.5 μ. This collection is the first report of a fungus on this annual introduced bent grass from the west, and possibly from anywhere.

I am indebted to several collectors, but especially to G. W. Fischer and Jack P. Meiners for aid in gathering the material for study, and to Prof. Frank Potter, Emeritus Professor of Philosophy, W. S. C., for revising the Latin descriptions. I appreciate the assistance of the Editor, Alexander Smith, who has made editorial suggestions.

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STUDIES IN THE LOWER CHYTRIDIALES. II. ENDO-OPERCULATION AND SEXUALITY IN THE GENUS DIPLOPHLYCTIS[1]

R. H. Haskins[2]

(WITH 10 FIGURES)

It is almost inevitable that in any extensive investigation (Haskins and Weston, **1950**) of a group of organisms as diverse and as little known as are the lower Chytridiales, new and interesting things will be encountered. This was especially true in studies made on the Diplophlyctis-Nephrochytrium group of chytrids. On the basis of operculation, Sparrow (**1943**) has rather widely separated the inoperculate genus *Diplophlyctis* from the operculate genus *Nephrochytrium*. These two genera are alike in many ways, and hence confusion has arisen with the description of chytrids similar in more respects to *Diplophlyctis* than to *Nephrochytrium*, yet assigned to the latter genus because of the presence of "endo-opercula" (Karling, **1944**). Investigations described in the present series of studies have revealed that there exist forms that may be either inoperculate or "endo-operculate."

Haskins (**1939**) mentioned a species of *Diplophlyctis* which was considerably coarser and larger than *Diplophlyctis intestina* (Schenk) Schroeter. This new species followed closely much of Karling's (**1928, 1930, 1936**) developmental description for *D.*

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[1] This paper covers a portion of a dissertation presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Harvard University in 1948.

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intestina particularly in regard to variation in shape of zoösporangia, size of zoöspores, and morphology and germination of the resting structures. Further studies of this *Diplophlyctis* in connection with the present work have yielded additional information, for in addition to its larger size, the organism differs somewhat in thallus development, in the occasional presence of so-called "endo-opercula," and the presence of sexually-formed resting spores.

As for size, the spherical sporangia extend up to over 170 μ in diameter, though the dimension in one direction may exceed this in irregular zoösporangia. The thallus appears very coarse when compared with the delicate thallus of *D. intestina* growing in *Nitella* internodes. The exit tubes, one or several, may be very short and broad, with reflexed rims, or may be long and narrow, extending up to 150 μ from the main body of the zoösporangium.

In the development of the thallus there is a deviation from the sequence of events described by Karling (**1930**) for *D. intestina* and later confirmed by Sparrow (**1936**). According to their observations, the apophysis is laid down after the rudiment of the sporangium is formed. In *Nephrochytrium* the sporangium forms as a bud from the wall of the apophysis. In the present species, the sequence is as follows: at the end of a branch (usually a short lateral one) of a germ tube from the encysted zoospore, a tiny more or less dumb-bell-shaped swelling appears. The distal part of this swelling enlarges more rapidly than the proximal part (FIG. 1), and becomes the rudiment of the zoösporangium, while the proximal swelling enlarges to become the apophysis (FIGS. 2–4). The apophysis, then, appears at the same time as the sporangium. The connection or isthmus between the apophysis and the sporangium is always broad and has not been observed to be narrow as with *D. intestina* or with the species of *Nephrochytrium.*

The presence of so-called "endo-opercula" in this group has created considerable taxonomic uncertainty, similar to that to be described in a later paper of this series, for the *Rhizophlyctis*-etc. Group. *Nephrochytrium stellatum* Couch (Couch, **1938**) dehisces ". . . always with a distinct cap," as does *Nephrochytrium aurantium* Whiffen (Whiffen, **1941**). In *D. intestina* and *Diplophlyctis laevis* Sparrow the dehiscence is inoperculate. On the basis of the presence of variously located "endo-opercula," Karling (**1944**) has placed *Nephrochytrium amazonensis* Karling in the genus *Nephrochytrium,* at the same time emphasizing its great similarity to the genus *Diplophlyctis.* Our species, in rapidly growing cultures, dehisces in a normal inoperculate manner and occasionally after extrusion of a gelatinous plug (FIG. 7), but in older stagnating cultures, after the deliquescence of the gelatinous tip of the exit-tube has occurred, the membrane between the protoplasmic content of the sporangium and the external medium thickens (FIG. 5). This membrane may thicken so as to form an endo-operculum-like structure (FIG. 8), which at subsequent dehiscence of the sporangium may merely rupture (FIG. 6) or may be forced out as a cap as described for *N. amazonensis* (Karling, **1944**).

A study of the formation of the resting spores of the present strain yielded interesting and important information. The size of the resting structure (16–23 μ) was found to be remarkably uniform, even when the organism was grown on media on which the vegetative thallus size varied greatly. This observation prompted a careful search for evidence of sexuality. This was soon found, but the sexuality was of a rather different type from that for which Sparrow (**1936**) had already presented evidence.

Though apparently normal resting spores were often found remote from other fungi on a piece of substrate, the majority were observed to be in groups of 2 to over 30 individuals. Examination of the smaller and more spread-out groups revealed the presence of small, thin-walled thalli which appeared to contain only the emptied cysts of zoöspores that had encysted in situ. From these cysts fine rhizoid-like germination tubes extended through the sporangial wall into the surrounding medium, where many of them were in open connection with the rhizoidal systems of fully developed resting spores (FIGS. 9–10).

When the group illustrated in figure 10 was first observed, the resting spore on the extreme left was hyaline, small, and obviously undeveloped with a thin, but faintly spiny wall. All of the cysts within what we may call the "male" thallus, were empty with the exception of the one which was connected with the above-mentioned rudimentary resting spore. The contents of this cyst in a few hours had entered the connecting tube where the large refractive globule caused a local distension of the tube. This globule was observed to move slowly along the tube towards the resting structure and within about an hour had entered its apophysis. Observation had to be discontinued, but by the following day the globule was no longer in the apophysis, and the resting spore had become a brown, thick-walled, spiny resting structure. The material at this point was killed and fixed. Cytological studies of this phenomenon will be undertaken to provide supporting evidence that a sexual fusion has actually occurred.

The rhizoidal connections of the cysts within the thin-walled "male" thalli to the resting spores have been observed many times. Single normal resting spores have been found remote from all other thalli, and these have germinated normally, functioning as prosporangia to produce single sessile, thin-walled zoösporangia. This indicates that resting spores may also be formed asexually. Supporting the foregoing evidence for the presence of sexuality in this new species, are "male" thalli with contents in the cysts, but in connection with no other thalli—these have not been observed to develop further other than to produce the rhizoidal germination tubes (FIGS. 9–10). In addition to these isolated "male" thalli, are found isolated, small, hyaline, thin-walled, minutely-spiny, undeveloped resting spores, that also have never been observed to develop further presumably because rhizoidal outgrowths from the "male" thalli failed to reach them.

Because of the coarseness and larger size of the thalli, the manner of development of the thallus, the broad isthmus between the apophysis and the sporangium, the inoperculate but occasionally "endo-operculate" nature of the sporangium, and the type of sexuality, this organism is believed to be a species of *Diplophlyctis* other than *D. intestina* (Schenk) Schroeter and *D. laevis* Sparrow, and therefore is considered new. It is characterized by its relative coarseness and its hitherto undescribed type of sexuality, and is accordingly named *Diplophlyctis sexualis.*

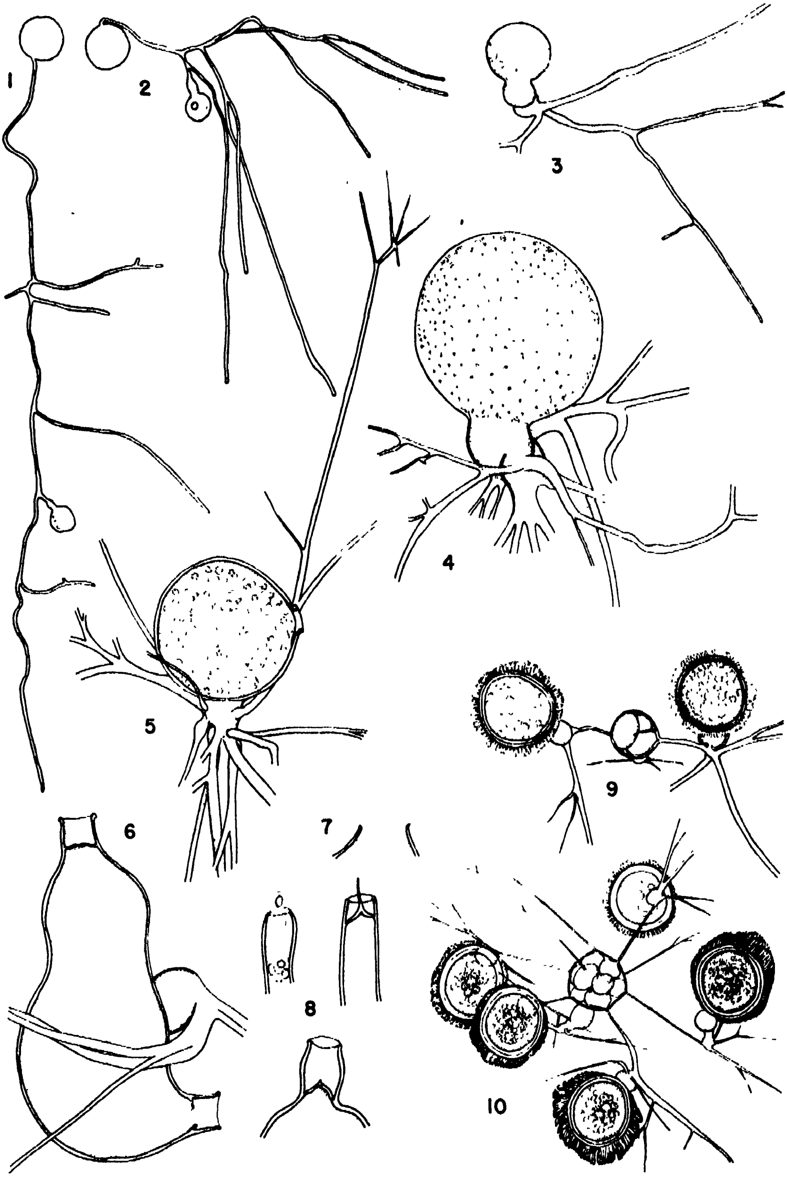
Diplophlyctis sexualis n. sp.

Zoösporangiis plerumque intramatricalibus, laevibus, hyalinis, globosis vel variatim formatis, 50–170 μ. Tubulis exeuntibus 1–5, brevibus rarius elonga-

tis, 10–150 μ, obturaculo gelatinoso, interdum cum endooperculis. Zoosporis globosis vel subglobosis, 5–6 μ, cum globulo uno refractivo, 2–4 μ. Vesiculo subsporangiali cum zoösporangio in isthmo amplo jugato, oriente uno atque eodem tempore quam sporangio. Rhizoidibus robustis. Sporis perdurantibus globosis, 16–23 μ, membrana crassa echinulataque, sexualiter vel asexualiter orientibus. Aplanogametibus masculis encystantibus in situ, i.e., in gametangio, copulatio per rhizoidales anastomosas. Sporis perdurantibus prosporangio vice in germinatione fungentibus ut surgat per poram exiguum zoösporangium extramatricale evanescens.

Asexual thallus monocentric, usually intramatrical, consisting of a sporangium or resting spore subtended by an apophysis from which arises a somewhat coarse, branched rhizoidal system. Sporangia hyaline, smooth, spherical, 50–170 μ in diameter, or variously shaped, walls not staining with zinc chloroiodide. Exit tubes one to several, short and broad, or occasionally very long up to 150 μ. Dehiscence by deliquescence of tips of exit-tubes or by tip of tube softening to form an evanescent gelatinous plug beneath which an "endo-operculum" may or may not develop. Endo-opercula, when formed, shallow saucer-shaped, to cone-shaped with long spine. Zoöspores spherical to sub-spherical, 5–6 μ in diameter each with single, large, prominent, highly refractive globule and a 30–40 μ flagellum, remaining temporarily motionless immediately after discharge, intermittently amoeboid. Apophysis spherical or variously shaped, joined to sporangium by a wide isthmus. Sporangium and apophysis originating at the same time at the end of an usually short branch of the germination tube from the encysted zoöspore. Rhizoidal system usually arising from basal half of apophysis, stout, extensive, and much branched. Resting spores spherical, 16–23 μ in diameter; contents coarsely globular; wall thick, layered, dark brown, densely spiny, spines short and stout to long and hair-like; formed asexually or only after sexual fusion. Female thalli as for resting spores and remaining abortive unless fertilized. Male thalli consisting of a gametangium, apophysis and rudimentary sparsely-branched rhizoidal system. Gametangium hyaline, thin-walled containing 4 to many gametes. Gametes encysting in situ producing fine sparsely-branched rhizoid-like germination tubes which penetrate gametangium wall to anastomose with rhizoidal system of female thallus, through which anastomosis the content of the male cell passes to enter the female cell, which immediately develops into normal, brown, spiny-walled resting spore. Resting spores functioning as pro-sporangia in germination, each producing one sessile, thin-walled zoösporangium.

Saprophytic in natural habitat in decaying vegetable débris, readily developing on boiled leaves of maize, Cellophane, and lens paper



FIGS. 1–10.

submerged in water; collected in London, Ontario, Canada; Rensselaerville (Albany County), New York; and Cambridge, Mass., U. S. A.

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EXPLANATION OF FIGURES

FIGS. 1–2, Early stages in thallus development showing formation of rudiments of apophysis and enlarging sporangium. In earlier stages these are equal in size (× 625). FIGS. 3–5, Portions of maturing thalli (× 225). FIG. 6, Emptied zoösporangium showing walls ("endo-opercula") formed across bases of exit tubes under certain conditions (× 188). FIG. 7, Tip of exit tube showing gelatinous plug sometimes present in the tube (× 188). FIG. 8, Formation of operculum-like structures ("endo-opercula") within exit tubes, the tips of which have already deliquesced (× 188). FIG. 9, Two resting spores in connection with a male thallus (× 500). FIG. 10, Five mature resting spores in connection with cysts enclosed within a single male thallus (× 500).

(All figures drawn with aid of a Spencer Camera Lucida.)

UREDINALES OF CONTINENTAL CHINA COLLECTED BY S. Y. CHEO. I[1]

GEORGE B. CUMMINS

(WITH 10 FIGURES)

The rusts reported in this paper were collected in cooperation between the Farlow Herbarium of Harvard University and the University of Nanking. All specimens are deposited in the Arthur Herbarium, Purdue University, and the Farlow Herbarium. Species preceded by an asterisk have not been recorded previously from continental China, insofar as I have been able to ascertain.

In general a conservative attitude has been adopted with respect to identifications; consequently only a few species have been described as new. This policy was followed because of the meager amount of Chinese material available for comparative study and because of uncertainty, in some cases, concerning the identity of the host material of Cheo's collections. With respect to both specimens and literature I have had the valuable assistance of Dr. Lee Ling of the United Nations Food and Agricultural Organizations as well as access to the rust specimens in the Chinese National Herbarium, now deposited in the Mycological Collections of the United States Department of Agriculture.

PUCCINIASTRUM AGRIMONIAE (Diet.) Tranz. On *Agrimonia* sp.: KWANGSI: Yung Hsien, Aug. 1933, (*2588*); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*384*).

PUCCINIASTRUM POTENTILLAE Kom. On *Fragaria* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1155*); KIANGSI: Sin Tsz Hsien, Sept. 1932, (*1046*).

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[1] Cooperative investigations between the Purdue University Agricultural Experiment Station and the Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture. Journal Paper Number 450, of the Purdue University Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology. The first paper, based on the Cheo collections and covering the species of *Puccinia* on the Lauraceae, was published in Bull. Torrey Bot. Club **76**: 31–33. 1949.

Only uredia are present but they agree well with those of *P. potentillae,* a rust previously recorded on *Potentilla fragarioides.* The specimen consists of trifoliate leaves having the appearance of *Fragaria,* but it is possible that the host is a species of *Potentilla.*

*PUCCINIASTRUM HYDRANGEAE-PETIOLARIDIS Hirats. f. On *Hydrangea* sp.: KWEICHOW: Chiang K'ou Hsien, Nov. 1931, (*872*).

PUCCINIASTRUM CORIARIAE Diet. On *Coriaria sinica* Miq.: KWEICHOW: Tsunyi Hsien, Aug. 1931, (*284*). On *Coriaria* sp.: KWANGSI: Ling Yuin Hsien, Mar. 1933, (*1730*).

PUCCINIASTRUM TILIAE Miyabe. On *Tilia tuan* Szysz: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*521*).

PUCCINIASTRUM CASTANEAE Diet. On *Castanea* sp.: ANHWEI: Ch'ing Yung Hsien, Oct. 1932, (*1385*); KWEICHOW: Tsunyi Hsien, Aug. 1931, (*273*).

*MELAMPSORIDIUM CARPINI (Fuckel) Diet. On *Carpinus* sp.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*606*).

MELAMPSORA KUSANOI Diet. On *Hypericum patulum* Thunb.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*487*), Tsunyi Hsien, July 1931, (*150*). On *Hypericum* sp.: KWANGSI: Ling Yuin Hsien, Mar., Apr. 1933, (*1607, 1609, 1664, 1956*).

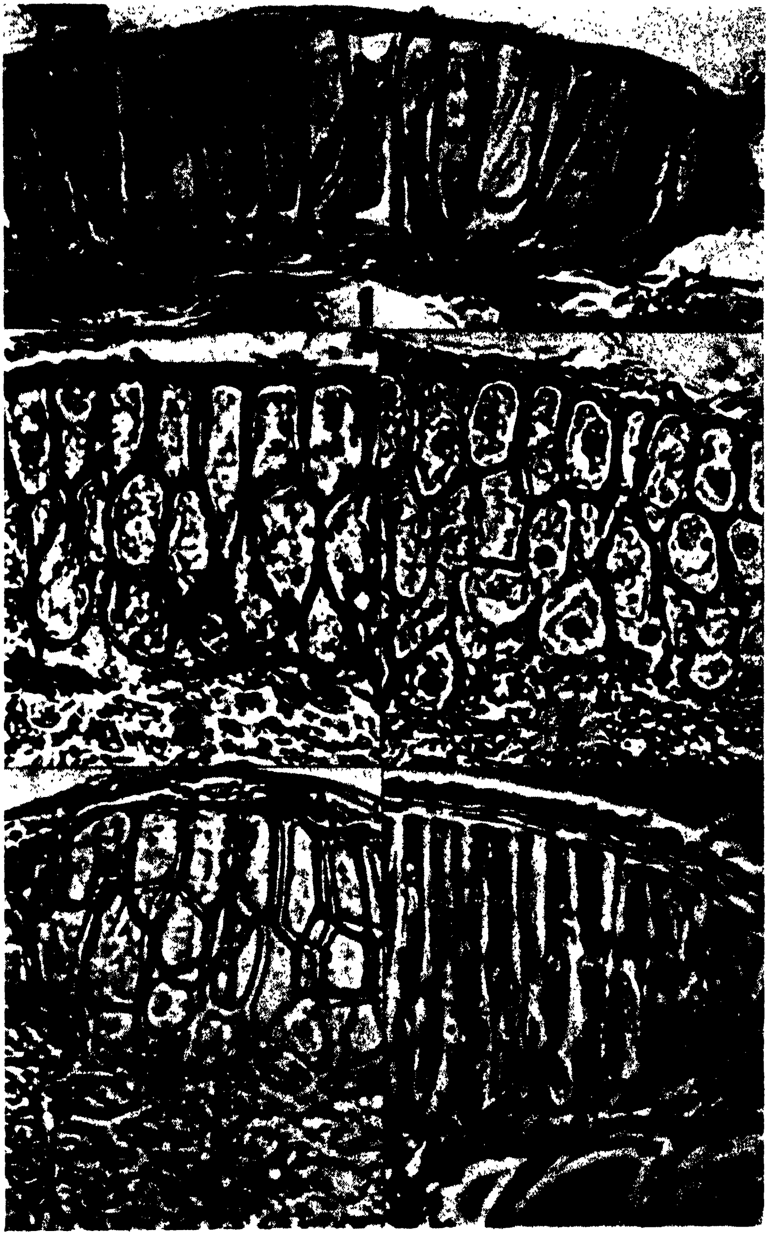
MELAMPSORA EUPHORBIAE-DULCIS Otth. On *Euphorbia chrysocoma glaucophylla* Levl.: KWEICHOW: Chiang K'ou Hsien, Oct. 1931, (*656*). On *Euphorbia pekinensis* Rupr.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*1022*). On *Euphorbia* sp.: ANHWEI: Ch'ing Yang Hsien, Nov. 1932, (*1501*).

MELAMPSORA EUPHORBIAE (Schub.) Cast. On *Euphorbia* sp.: KWANGSI: Ling Yuin Hsien, June 1933, (*2216*), Lo Ch'en, Oct. 1933, (*2871*).

In Cheo's collections telia of *M. euphorbiae-dulcis* are present but only uredia of *M. euphorbiae.* The latter determination, consequently, is open to question.

*MELAMPSORA YEZOENSIS Miyabe & Matsumoto. On *Salix longiflora* Anders.: KWEICHOW: Tsunyi Hsien, Aug. 1931, (*263*), Chiang K'ou Hsien, Sept. 1931, (*370*a).

No material of *M. yezoensis* has been available for comparison but telia are epiphyllous and subcuticular, with teliospores measuring 7–12 × 23–35 μ and with the apical wall thin. The uredio-



FIGS. 1–5. Telia of *Melampsora* and *Phakopsora*.

spores have walls 3–6 μ in thickness against 3–10 μ, as given in the description of the species. The thickness of the urediospore wall separates this rust from *M. ribesii-viminalis* Kleb. although the telia are apparently similar. The latter species develops aecia on *Ribes* while the aecial host of *M. yezoensis* is *Corydalis.*

***Melampsora aleuritidis** sp. nov.

Spermagoniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, sparsis vel aggregatis, plus minusve rotundatis, 0.2–0.5 mm. diam., flavidis, pulverulentis; paraphysibus plerumque capitatis, 12–19 × 40–55 μ, membrana hyalina, 1.5–2 μ crassa; urediosporis late ellipsoideis vel obovatis, 14–18 × 18–24 μ; membrana hyalina, 2–2.5 μ crassa, moderate echinulata; poris germ. obscuris. Teliis (FIG. 1) hypophyllis, subepidermalibus, denseque aggregatis vel confluentibus, rotundatis, 0.1–0.4 mm. diam., aureo- vel castaneo-brunneis; teliosporis oblongis vel prismaticis, 8–13 × 31–43 μ; membranis ubique 1–1.5 μ crassis, hyalinis vel pallide brunneis; statim germ.

On *Aleurites* sp.: KWEICHOW: Fan Ching Shan, Chiang K'ou Hsien, Oct. 19, 1931, *S. Y. Cheo 730* (type!).

This is the first species of *Melampsora* reported as parasitizing *Aleurites* and is interesting, in addition, because the teliospores germinate without a period of dormancy.

MELAMPSORA LARICI-EPITEA Kleb.? On *Salix* sp.: ANHWEI: Ch'ing Yang Hsien, Nov. 1923, (*1433, 1489, 1500, 1518, 1589*); KWEICHOW: Tsunyi Hsien, July 1931, (*56*), Chiang K'ou Hsien, Sept. 1931, (*370*).

Most of these collections have both uredia and telia, the latter (FIG. 5) subcuticular with long narrow spores (4–9 × 40–55 μ) whose apical wall is not thickened.

While *M. larici-epitea* was described as having subepidermal telia Matsumoto (Trans. Sapporo Nat. Hist. Soc. **6**: 11. 1915) includes both subcuticular and subepidermal rusts. It is extremely doubtful if such a procedure is correct and, consequently, none of Cheo's collection having telia (all except No. 370) probably represents this species. On the other hand agreement is poor with named subcuticular species because of the long, narrow, non-thickened teliospores.

Without adequate Asiatic *Salix* rusts for comparison the collections are arbitrarily assigned to *M. larici-epitea,* a rust recorded as occurring in China on three species of *Salix.*

MELAMPSORA COLEOSPORIOIDES Diet. On *Salix mesneyi* Hance: KIANGSI: Hsing Tsu Hsien, Sept. 1932, (*1009*).

*CHNOOPSORA ITOANA Hirats. f. On *Oxalis* sp.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*414*).

*Phakopsora malloti sp. nov.

Spermogoniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, minutis, 0.1–0.2 mm. diam., brunneis; peridiis hemisphaericis cum paraphysibus apicalis praeditis, paraphysibus incurvatis, 13–18 μ latis et usque ad 40 μ longis, membrana 1 μ, ad apicum 3–5 μ crassa; urediosporis subglobosis vel obovatis vel ellipsoideis, 16–20 (–22) × (21–) 24–32 (–35) μ; membrana 1.5 μ crassa, pallide flavida vel brunnea, minuteque echinulata; poris germ. obscuris sed 4 vel 5, equatorialibus. Teliis (FIG. 3) hypophyllis, subepidermalibus, sparsis vel aggregatis, atro-brunneis, rotundatis, 0.2–0.5 mm. diam. vel confluentibus, ex sporis 2-6 irregulariter superpositis compositis; teliosporis variabilis, cuboideis, oblongis, vel plus minusve fusoideis, 8–13 × 13–30 μ; membrana aureo-brunnea, ubique 1–1.5 μ vel ad apicum usque ad 2 μ crassa.

On *Mallotus* sp.: KWEICHOW: Fan Ching Shan, Chiang K'ou Hsien, Oct. 26, 1931, *S. Y. Cheo 786* (type!).

If one follows Hiratsuka's treatment of the Japanese species of *Phakopsora* (Bot. Mag. Tokyo **49**: 781–788. 1935), *P. malloti* would belong in the section *Physopella,* in which the uredia have incurved paraphyses and often also a basal peridium. There is no presently named species which should cause confusion. Hiratsuka (Bot. Mag. Tokyo **49**: 23. 1935) has described *Pucciniastrum malloti* on *M. japonicus* from Formosa and based on uredia only. Through the courtesy of Dr. Ling I have seen some of this material, collected by Hashioka, and find that the peridium has apically free units and brownish spores. It may prove to be synonymous with the species named here.

PHAKOPSORA FORMOSANA Syd. On *Glochidion* sp.: KWANGSI: Ling Yuin Hsien, May 1933, (*2094*).

Only the aecial stage is present and it appears to be identical with *Aecidium innatum* Syd. & But! *P. innata* Sawada is doubtless synonymous.

*PHAKOPSORA FICI-ERECTI Ito & Otani (FIG. 2). On *Ficus* sp.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*961*). On *Morus alba* L.: KIANGSI: Sin Tsz Hsien, Sept. 1932, (*1050*).

Recently, Tai (Farlowia **3**: 98. 1947) described *P. hengshanen-*

sis on *Ficus martini* and *F. heteromorpha* from Hunan Province. This species has not been available for study but, from the description, it could well be synonymous with *P. fici-erecti.* I have seen a rust on *Ficus beecheyana* from Formosa which appears to be the same as Cheo's collections. It has been determined by Sawada as *P. nishidana* but Hiratsuka (Mem. Tottori Agric. Coll. **7**: 12. 1943; **7**: 208. 1944) assigns the Formosan material to *P. fici-erecti* while recognizing *P. nishidana* as a separate species occurring on *F. carica.* He cites, as hosts of *P. fici-erecti, F. beecheyana, F. erecta, F. erecta* var. *sieboldii, F. formosana,* and *F. vasculosa.* Neither Tai nor Hiratsuka cites *Morus alba* as a host. A careful study, with adequate material, of these species would be desirable and should take into account the rust usually passing as *Cerotelium fici* (Butl.) Arth.

***Phakopsora cheoana** sp. nov.

Spermogoniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, brunneis, usque ad 120 μ diam., paraphysibus periphericis, incurvatis, plus minusve capitatis, 8–12 × 30–45 μ; membrana in partim ventralis 1 μ, in partim dorsalis et apicalis 3–5 μ crassa. Urediosporis late ellipsoideis, 15–18 × 22–26 μ; membrana 1–1.5 μ crassa, minuteque echinulata, flavida vel hyalina; poris germ. obscuris. Teliis (FIG. 4) hypophyllis, subepidermalibus, sparsis vel dense aggregatis, atro-brunneis, rotundatis, usque ad 200 μ diam. vel plus minusve confluentibus, ex sporis 2 vel 3 superpositis compositis; teliosporis oblongis vel plus minusve cubicis, 6–10 × 10–23 μ; membrana aureis vel pallide brunneis, ubique 1.5 μ crassa.

On *Cedrela sinensis* Juss.: KWEICHOW: Fan Ching Shan, Chiang K'ou Hsien, Oct. 27, 1931, *S. Y. Cheo 789* (type!).

There has been no species of *Phakopsora* described as occurring on the family Meliaceae. Since the uredia are old in this material and few spores are present it is possible that the description of the urediospores is not accurate.

*PHAKOPSORA MEIBOMIAE Arth. On *Desmodium racemosum* (Thunb.) DC.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*575*).

PHAKOPSORA PACHYRHIZAE Syd. On *Glycine sojae* Benth.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1224*); KIANGSI: Sin Tsz Hsien, Sept. 1932, (*1084*); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*356*). On *Glycine* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1231*). KWANGSI: San Kiang Hsien, Sept. 1933, (*2697*). On *Pueraria thunbergiana* Benth.: KIANGSI: Sin Tsz Hsien, Sept. 1932, (*1056*); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*368*). On *Shuteria* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (*2763*).

The rust on *Shuteria* is present as uredia and thus is only tentatively referred to this species.

*PHAKOPSORA MELIOSMAE Kusano. On *Meliosma oldhami* Miq.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1274*). On *Meliosma simplicifolia* Roxb.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1250*). On *Meliosma* sp.: KWEICHOW: Fan Ching Shan, Oct. 1931, (*794*).

Thirumalachar and Kern (Mycologia **41**: 288. 1949) have transferred this species to *Angiopsora* since they consider that the spores are catenulately produced.

PHAKOPSORA ZIZYPHI-VULGARIS Diet. On *Zizyphus jujuba* Mill. var. *inermis* (Bge.) Rehd.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*497*). On *Zizyphus sativus* Gaertn.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1347*). On *Zizyphus* sp.: KWANGSI: Lo Ch'en, Oct. 1933, (*2875*).

PHAKOPSORA AMPELOPSIDIS Diet. & Syd. On *Ampelopsis* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1363*). On *Vitis pentagona* Diels & Gigl.: KWEICHOW: Sze Hsien, Aug. 1931, (*344*), Chiang K'ou Hsien, Sept. 1931, (*390, 506*). On *Vitis* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1169, 1179, 1380*); KWANGSI: Yung Hsien, Oct. 1933, (*2893*).

This rust has been recorded on *Ampelopsis japonica, Vitis betulifolia,* and *V. vinifera* in China. Both uredia and telia are present on most of the above collections.

In 1935, Hiratsuka (Bot. Mag. Tokyo **49**: 857) considered the common grape rust to be the same as the *Phakopsora* on *Ampelopsis,* thus reducing to synonymy *Uredo vitis* Thuem. and telial names based on it. This is undoubtedly correct since all specimens seen with telia, including American material, seem identical. Thirumalachar and Kern (Mycologia **41**: 288. 1949) consider this species to be an *Angiopsora* and have, consequently, made the transfer.

PHAKOPSORA PUNCTIFORMIS (Barcl. & Diet.) Diet. On *Galium aparine* L.: KWEICHOW: Tsunyi Hsien, Aug. 1931, (*199*).

PHAKOPSORA ARTEMISIAE Hirats. f. On *Artemisia* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1145, 1159*); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*584, 613*). On *Aster* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1255*); KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*1027*). On *Chrysanthemum* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1332*).

Another species, *Phakopsora compositarum* Miyake, occurs in China but is not represented in Cheo's collections. Both species are recognized by Hiratsuka (*l.c.*). *P. artemisiae* has mostly epiphyllous uredia, urediospores 24–36 μ long, and teliospores 19–33 μ long while in *P. compositarum* the uredia are hypophyllous, the urediospores 24–28 μ long, but the teliospores 24–60 μ in length. The latter species has not been available to me.

***Phakopsora incompleta** (Syd.) comb. nov. (*Puccinia incompleta* Syd. Annal. Mycol. **10**: 261. 1912). On *Andropogon* sp.: KWANGSI: Yung Hsien, Oct. 1933, (*2904*). On *Eulalia* sp.: KWANGSI: Ta Tseh Tsuen, Aug. 1933, (*2311, 2420*).

This rust, originally described from India on *Ischaemum* and subsequently reported for Formosa and the Philippines, apparently also occurs in Africa on *Andropogon* and possibly *Exotheca.* In addition to the African specimens I have seen two Indian collections, including the type. While the uredia and urediospores are essentially alike in all specimens the only telia that I have seen are in Cheo's Nos. 2311 and 2904. Sydow reported seeing only two immature teliospores but gave no measurements nor did he mention pedicels.

Since, from specimens now available, it is apparent that the rust is not a species of *Puccinia,* the species is transferred to *Phakopsora* with the following description.

Uredia hypophyllous, scattered, minute, 0.1–0.2 mm. diam., with abundant peripheral, incurved, hyaline or pale golden paraphyses, 8–13 × 35–45 μ, the outer (convex) wall 2–4 μ thick, the inner (concave) wall 1–1.5 μ thick; urediospores obovoid, ellipsoid, or broadly ellipsoid, 15–19 × 19–26 μ, wall pale brownish to nearly hyaline, finely and closely echinulate, 1–1.5 μ thick, pores probably scattered, obscure. Telia mostly hypophyllous, minute, oval or ellipsoid, 0.2–0.5 mm. long, chestnut-brown, subepidermal and indehiscent, 1–3 spores in thickness; teliospores irregularly arranged, variable, more or less oblong, 8–15 × 18–26 (–35) μ; wall golden or pale brownish, uniformly 1–1.5 μ thick, smooth.

CRONARTIUM QUERCUUM Miyabe. On *Quercus* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (*2789*); KWEICHOW: Sze Nan Hsien, Aug. 1931, (*351*), Chiang K'ou Hsien, Sept. 1931, (*609*).

COLEOSPORIUM CLEMATIDIS Barcl. On *Clematis grata* Wall. var. *grandidentata* Rehd. & Wils.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*403*). On *Clematis montana* Buch.-Ham.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*616*). On *Clematis* sp.: ANHWEI: Ch'ing Yang Hsien, Nov. 1932, (*1541*); KIANGSI: Hsing Tzu Hsien, Nov. 1932, (*924*); KWANGSI: San Kiang Hsien, Sept. 1933, (*2769, 2809, 2861*); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*408, 395, 535*), Tungjen Hsien, Dec. 1931, (*891*), Tsunyi Hsien, July 1931, (*105, 161*).

COLEOSPORIUM EVODIAE Diet. On *Evodia meliaefolia* Benth.: KWEICHOW: Sze Nan Hsien, Aug. 1931, (*347*), Tsunyi Hsien, July 1931, (*46*). On *Evodia officinalis* Dode: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1190*). On *Evodia* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1286, 1382*); KWANGSI: Ling Yuin Hsien, Mar. 1933, (*1644*), Yung Hsien, Aug. 1933, (*2305*), Sze Nan Hsien, Aug. 1931, (*347*).

COLEOSPORIUM XANTHOXYLI Diet. & Syd. On *Zanthoxylum* sp.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*489*).

*Coleosporium violae** sp. nov.

Spermagoniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, flavidis, plus minusve pulverulentis, 0.1–0.5 mm. diam.; urediosporis globoideis vel late ellipsoideis, 13–18 × 17–24 μ; membrana verrucosa, hyaline, 1.5–2 μ crassa; poris germ. obscuris. Teliis hypophyllis, sparsis, rotundatis, 0.1–0.4 mm. diam. vel confluentibus, ceraceis, pallide aureis; teliosporis cylindraceis, utrinque rotundatis, 13–20 × 43–56 μ; episporio hyalino, 1 μ crasso, ad apicem 8–15 μ et valde gelatinoso; statim germ. Basidiosporis non visis.

On *Viola limprechtiana* W. Becker: KWEICHOW: Ling Feng Yah, Tsunyi Hsien, Aug. 11, 1931, *S. Y. Cheo 289* (type!).

Species of *Coleosporium* have not been collected on Violaceae previously.

***Coleosporium cheoanum** sp. nov.

Spermogoniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, sparsis, flavidis, rotundatis, 0.1–0.4 mm. diam., pulverulentis, urediosporis late ellipsoideis vel globoideis, 14–18 × 16–21 μ; membrana verrucosa, hyaline, 1.5–2 μ crassa; poris germ. obscuris. Teliis hypophyllis, subepidermalibus, rotundatis, 0.1–0.5 mm. diam., ceraceis, aureis; teliosporis cylindraceis, utrinque rotundatis, 13–19 × 32–52 μ; membrana hyalina, 1 μ crassa, ad apicem 3–8 μ, lamina gelatinosa inconspicue vel vix praedita; statim germ., basidiosporae 10–14 × 14–18 μ.

On *Coleus* sp.: KWEICHOW: Fan Ching Shan, Chiang K'ou Hsien, Sept. 21, 1931, *S. Y. Cheo 548* (type!).

C. cheoanum differs from other species which parasitize the Labiatae because of the small urediospores and teliospores. In addition the apical wall of the teliospores is unusually thin and shows little or no gelatinization.

***Coleosporium rubiicola** sp. nov.

Spermogoniis et aeciis ignotis. Urediis hypophyllis, sparsis, flavidis, rotundatis, 0.5–1.0 mm. diam., pulverulentis; urediosporis late ellipsoideis vel oblongo-ellipsoideis vel oblongis, 13–20 × 23–34 (–38) μ; episporio hyalino, verrucoso, 2 μ crasso; poris germ. obscuris. Teliis hypophyllis, subepidermalibus, ceraceis, aureis, rotundatis, 0.3–1.0 mm. diam., sparsis; teliosporis cylindraceis, utrinque rotundatis vel deorsum attenuatis, 15–19 × 100–125 μ; episporio hyalino, 1 μ crasso, ad apicem gelatinoso 12–20 μ crasso; statim germ.

On *Rubia cordifolia* L.: KWEICHOW: Fan Ching Shan, Tungjen Hsien, Nov. 6, 1931, *S. Y. Cheo 810* (type!).

Of the species parasitizing Rubiaceae *C. rubiicola* has the longest urediospores and teliospores. The teliospores are among the longest described in *Coleosporium*. *C. knoxiae* Syd. has nearly as long teliospores, 65–100 μ, but the urediospores are smaller, 15–20 × 17–25 μ. I have been unable to find a previous report of a *Coleosporium* on the genus *Rubia*.

COLEOSPORIUM PLECTRANTHI Barcl. On *Plectranthus racemosus* Hemsl.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*898*). On *Plectranthus* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1299*); KWANGSI: San Kiang Hsien, Sept. 1933, (*2844*).

COLEOSPORIUM PERILLAE Syd. On *Mosla chinensis* Maxim.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1180*). On *Mosla* sp.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*928*); KWEICHOW:

Chiang K'ou Hsien, Sept. 1931, (*494*), Tsunyi Hsien, July, Aug. 1931, (*4, 191*). On *Perilla ocymoides* L.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1111*); KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*896*). On *Perilla* sp.: KIANGSI: Sin Tsz Hsien, Sept. 1932, (*1039*); KWANGSI: Yung Hsien, Aug., Oct., 1933, (*2526, 2887*); KWEICHOW: Tsunyi Hsien, July 1931, (*121*), Sze Nan Hsien, Aug. 1931, (*336*).

COLEOSPORIUM PAEDERIAE Diet. On *Paederia* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (*2806*); KWEICHOW: Sungtao Hsien, Nov. 1931, (*815*).

COLEOSPORIUM CAMPANULAE Lév. On *Adenophora* sp.: KIANGSI: Sin Tze Hsien, Sept. 1932, (*1044*). On *Platycodon* sp.: KWEICHOW: Fan Ching Shan, Sept. 1931, (*355*). On *Lobelia pyramidalis* Wall.: KIANGSI: Sin Tsz Hsien, Sept. 1932, (*1058*). On *Lobelia* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (*2728*); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*457*).

COLEOSPORIUM ASTERUM (Diet.) Syd. On *Aster* sp.: ANHWEI: Ch'ing Yang Hsien, Oct., Nov., 1932, (*1252, 1441, 1542, 1552*); KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*1029*); KWANGSI: Ling Yuin Hsien, June 1933, (*2290*), San Kiang Hsien, Sept. 1933, (*2821*); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*549*), Tsungi Hsien, July, Aug. 1931, (*49, 322*).

COLEOSPORIUM CARPESII Sacc. On *Carpesium* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1127*): KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*900*); KWANGSI: Ling Yuin Hsien, June 1933, (*2211*), Yung Hsien, Oct. 1933, (*2894*).

*COLEOSPORIUM ELEPHANTOPODIS Thuem. On *Elephantopus* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (*2860*).

COLEOSPORIUM EUPATORII Arth. On *Eupatorium lindleyanum* DC.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1240*). On *Eupatorium* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (*2696*); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*362, 397*).

COLEOSPORIUM SAUSSUREAE Thuem. On *Saussurea* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1217*); KWANGSI: San Kiang Hsien, Sept. 1933, (*2778*); KWEICHOW: Tsunyi Hsien, July, Aug. 1931, (*29, 228*).

*COLEOSPORIUM sp. On *Siegesbeckia orientalis* L.: KWEICHOW: Tsunyi Hsien, July 1931, (*34*).

I find no record of a species *Coleosporium* parasitizing *Siegesbeckia,* a genus of the tribe Heliantheae. Only uredia are present.

COLEOSPORIUM BLETIAE Diet. On *Anthogonum* sp.: KWEICHOW: Tsunyi Hsien, Aug. 1931, (*241*).

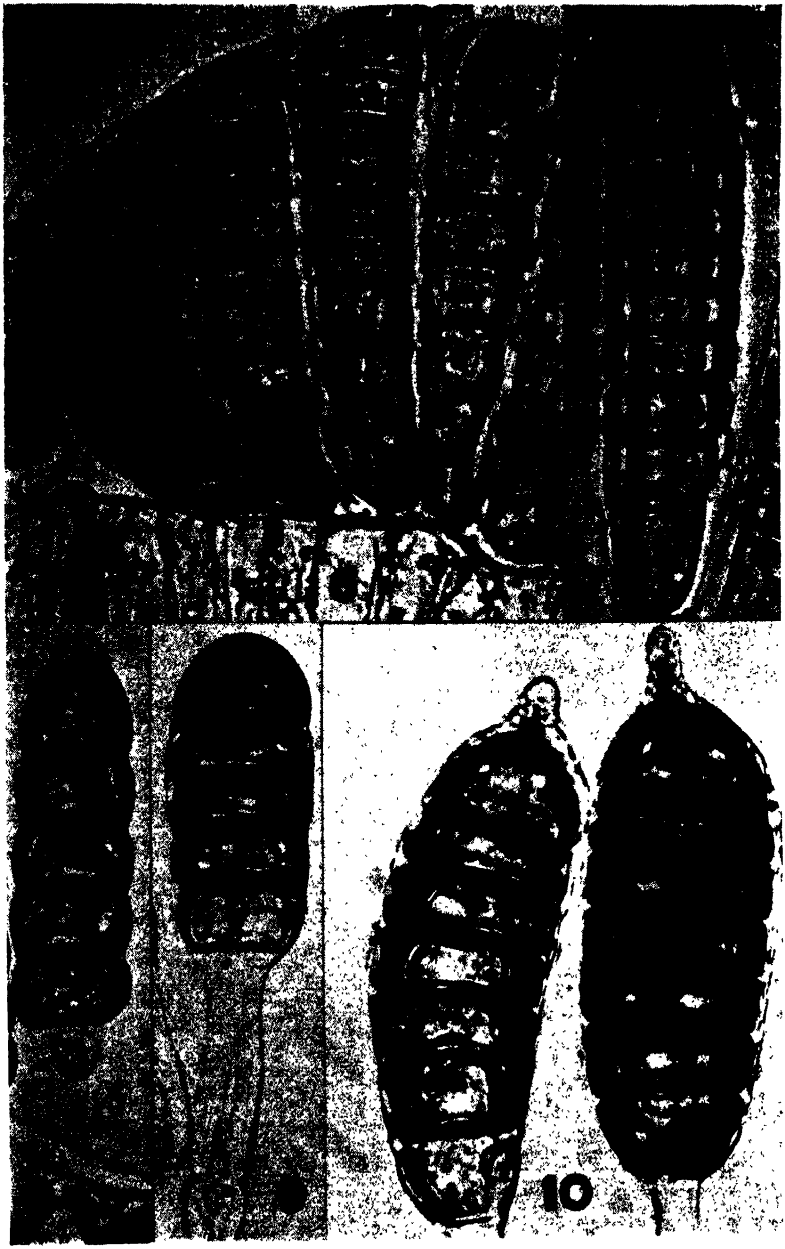
PUCCINIOSTELE MANDSCHURICA Diet. On *Astilbe chinensis* Franch. & Sav.: KWANGSI: San Kiang Hsien, Sept. 1933, (*2727*).

***Pucciniostele hashiokai** (Hirats. f.) comb. nov. (*Cerotelium hashiokai* Hirats. f., Jour. Jap. Bot. **13**: 248. 1937; *Pucciniostele ampelopsidis* Sawada, Formosan Agr. Rev. **38**: 703. 1942) (FIGS. 6, *7*). On *Ampelopsis* sp.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*514*); KWANGSI: Yung Hsien, Oct. 1933, (*2939*).

Through the courtesy of Y. Hashioka I was able to examine a Formosan specimen collected on *Ampelopsis cantoniensis* Planch. at Rengechi, Prov. Taichu, Nov. 3, 1932, by Hashioka. The type is Hashioka No. 218, collected at Gojyo, but both were cited in Hiratsuka's original description. The type of *Pucciniostele ampelopsidis* was furnished, together with a translation of Sawada's description, by Dr. Lee Ling. Sawada's specimen was collected at Urai on the same host. It is probable that the host of Cheo's collections is also *A. cantoniensis* and certainly the rust is the same in all collections.

While Hiratsuka's description is sufficiently accurate it is obvious that the rust is not a species of *Cerotelium* since each cell of the teliospore has two or three equatorially placed germ pores. While more difficult to observe this is apparently also true of *Pucciniostele clarkiana* (Barcl.) Diet. and *P. mandschurica.* In *P. hashiokai* the outer wall of the spore is somewhat hygroscopic, especially over the germ pores where it swells considerably (FIG. *7*). The spores do not adhere laterally and when mature give much the impression of a *Phragmidium* spore minus its pedicel.

The uredia are phakopsoroid in character with peripheral, incurved, dorsally thickened, septate paraphyses. With only uredia present it would be difficult accurately to distinguish this rust and *Phakopsora ampelopsidis* Diet. & Syd. While the two other species of *Pucciniostele* are described as lacking uredia I have found evidence, in Philippine specimens of *P. clarkiana,* that uredia occur in that species. Primary stages are unknown for *P. ampelopsidis.*



FIGS. 6–10. Telium of *Pucciniostele* and teliospores of *Pucciniostele* and *Phragmidium*.

*RAVENELIA ATRIDES Syd. On *Grewia parviflora* Bunge: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*964*).

This rust has not been recorded from China although it occurs in the Philippines. Telia have been found only in Africa, however.

*RAVENELIA INDIGOFERAE Tranz. On *Indigofera* sp.: KWANGSI: Ling Yuin Hsien, June 1933, (*2273*).

Only uredia are present, which makes the identification uncertain.

RAVENELIA JAPONICA Diet. & Syd. On *Albizzia* sp.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*929*); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*475*).

PILEOLARIA KLUGKISTIANA Diet. On *Rhus punjabensis* Stewart var. *nucipersica* (L.) Schneid.: KWEICHOW: Tsunyi Hsien, July 1931, (*127*). On *Rhus* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1114*); KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*1031*); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*509*).

PILEOLARIA SHIRAIANA (Diet. & Syd.) Ito. On *Rhus* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1105*); KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*1025*); KWANGSI: Yung Hsien, Aug. 1933, (*2459*), San Kiang Hsien, Sept. 1933, (*2695, 2815*); KWEICHOW: Tsunyi Hsien, Aug. 1931, (*260*).

These two species of *Pileolaria*, both previously recorded from China, are readily separable on the basis of either urediospores or teliospores. In *P. klugkistiana* the teliospores are deep chestnut-brown but not opaque, and the sculpturing consists of variously anastomosing ridges or warts which results in an irregularly and rather indistinctly reticulate pattern. The teliospores of *P. shiraiana* while immature are smoky or almost olivaceous brown, but with maturity become so densely pigmented and opaque that the thickness of the wall cannot be observed. There is no indication of reticulation but the wall is uniformly verrucose with somewhat conical warts.

Neither the number nor the location of the germ pores of the urediospores is given by the Sydows (Monogr. Ured. **3**: 145, 150. 1910) for either species. In *P. klugkistiana* the pores are four, as stated by Tai (Nanking Jour. **2**: 8. 1932), and equatorial, while in *P. shiraiana* there are two indistinct pores adjacent to the hilum. The wall in both species is spirally adorned with widely spaced ridges, homogeneous in structure in *P. klugkistiana* but beaded in *P. shiraiana*. Careful examination of the ridges of the latter species reveals that the ridges are actually continuous but with internal differentiation which, because of unequal refraction, gives a beaded appearance.

TRANZSCHELIA PRUNI-SPINOSAE (Pers.) Diet. On *Prunus persica* (L.) Stokes: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*904*); KWANGSI: Ling Yuin Hsien, May 1933, (*2102*). On *Prunus* sp.: KWANGSI: Ling Yuin Hsien, June 1933, (*2289*).

*KUEHNEOLA CALLICARPAE Syd. On *Callicarpa* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (*2766*).

GERWASIA CHINENSIS (Diet.) Hirats. f. On *Rubus amplifolius* Levl. & Van: KWEICHOW: Chiang K'ou Hsien. Oct. 1931, (*777*), Tungjen Hsien, Dec. 1931, (*890*). On *Rubus setchuensis* Wright: KWEICHOW: Tsunyi Hsien, July 1931, (*35*). On *Rubus* sp.: KWANGSI: Ling Yuin Hsien, Mar., Apr. 1933, (*1610, 1858, 1889*).

This appears to be a common Asiatic species. It remains a question how closely it may be related to *Gerwasia rubi* Racib., described from Java. No one has succeeded in obtaining Raciborski's type but recently Hiratsuka (Mem. Tottori Agric. Coll. **3**: 26. 1943) has reduced the above name to synonymy with *G. rubi*, which may well be correct. In this publication he does not include *G. fasciculata* Arth. & Cumm., although he had previously reduced it to synonymy under *G. chinensis*. Both species are superstomatal but *G. fasciculata* is paraphysate and doubtless a distinct species.

GERWASIA ROSAE Tai. On *Rosa roxburghii* Tratt.: KWEICHOW: Tsunyi Hsien, July 1931, (*86*). On *Rosa* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1384*); KWANGSI: Ling Yuin Hsien, Apr. 1933, (*1949*).

Only uredia are present in these collections and since Tai (Farlowia **3**: 103. 1947) described only telia the identification is uncertain. The uredia, however, correspond closely in structure to the telia described by Tai. The spores are produced at the apex of slender basal cells. The sori are at first superstomatal but later rupture the epidermis, and may attain a diameter of 100 μ or more. Incurved, unilaterally thickened paraphyses, borne in the same manner as the teliospores, are abundant. The paraphyses are one-septate, a characteristic not mentioned by Tai.

If these collections are not related to *Gerwasia* one would expect them to belong with an undescribed *Kuehneola*. There is, in the Philippines, a species of *Kuehneola*, thus far erroneously referred to *K. japonica*, which has uredinoid aecia whose spores are similar to the urediospores of Cheo's collections. Smaller, less abundant, non-septate, but otherwise similar paraphyses occur in these aecia. Telia are present in the three available specimens but no true uredia could be found. Presumably the species is an *opsis* type. Since *K. japonica* is microcyclic the Philippine rust cannot be identical; and since the Chinese specimens have uredia, smaller spores, and larger septate paraphyses they cannot be identified with the Philippine rust. A description of the uredia of the Chinese rust follows:

Uredia hypophyllous, usually closely grouped on brownish spots obvious on the upper side of the leaf, or the distribution more diffuse, minute, usually less than 100 μ, subepidermal or merely superstomatal when first formed, yellowish in dry condition; paraphyses abundant, incurved, hyaline or nearly so, 1-septate near the base, borne on basal cells with the urediospores, the wall 3–5 μ thick on the convex side, 1 μ on the concave side; urediospores ovoid, obovoid, or ellipsoid, usually with three slightly protruding angles in the equatorial zone, 16–19 (–21) × 21–26 (–29) μ; wall 1–1.5 μ thick, hyaline or pale yellowish, finely and closely echinulate, the pores obscure but probably 3, equatorial and located in the angles.

HAMASPORA ACUTISSIMA Syd. On *Rubus setchuensis* Wright: KWEICHOW: Sze Nan Hsien, Aug. 1931, (*350*). On *Rubus* sp.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (*957*); KWANGSI: Ling Yuin Hsien, Apr. 1933, (*1841, 1947*); KWEICHOW: Tsunyi Hsien, Aug. 1931, (*145*).

HAMASPORA BENGUETENSIS Syd. On *Rubus* sp.: ANHWEI: Ch'ing Yang Hsien, Nov. 1932, (*1435*); KWEICHOW: Tsunyi Hsien, Aug. 1931, (*220*).

Telia are not present in either collection. Since *H. benguetensis* and *H. rubi-sieboldii* (Kawagoe) Diet. differ mainly in teliospore morphology, identity of these collections is uncertain. *H. benguetensis* has been reported from China but *H. rubi-sieboldii* has not.

*HAMASPORA HASHIOKAI Hirats. f. On *Rubus* sp.: KWANGSI: Ling Yuin Hsien, Apr. 1933, (*1948*).

The most distinctive feature of this species is provided by the urediospores, which have a thickened apical wall. Reports of the occurrence of *H. longissima* (Thuem.) Koern. in China are probably erroneous and in part, at least, referable to *H. hashiokai.* There is, in the Arthur Herbarium, a specimen on *Rubus* sp. from Sikiang (*Teng 3294a*) which is certainly *H. hashiokai* and in Tai's "List" (Sci. Rept. Natl. Tsing Hua Univ. Ser. B. **2**: 297–418. 1937) *H. longissima* is recorded on *Rubus lambertianus,* which is the host of *H. hashiokai.*

HAMASPORA SINICA Tai & Cheo. On *Rubus* sp.: KWANGSI: Ling Yuin Hsien, Mar. 1933, (*1762*) (issued as *H. acutissima* Syd. in Reliq. Farlowianae No. 730), Apr. 1933, (*1822, 1825*).

A portion of the type has been available for comparison. There is considerable chance of confusing *H. sinica, H. taiwaniana* Hirats. f. & Hashioka, and *H. acutissima* since the number of septations and the degree of apical thickening of the teliospores vary considerably. In addition, uredia have not been described for *H. sinica* and *H. taiwaniana.*

PHRAGMIDIUM POTENTILLAE (Pers.) Karst. On *Potentilla* sp.: Ling Yuin Hsien, Mar. 1933, (*1729*).

PHRAGMIDIUM GRISEUM Diet. On *Rubus* sp.: ANHWEI: Ch'ing Yang Hsien, Oct., Nov. 1932, (*1123, 1466*).

The teliospores of this species (FIG. 8) are usually narrower than those of *P. rubi-thunbergii* (FIG. 9) and the apex abruptly narrowed and umbonate, with the wall of the umbonate portion subhyaline and thickened. Both species belong in the section *Phragmotelium,* which is frequently treated as a distinct genus. I am following Hiratsuka's concept as presented in his treatment of the Japanese species of *Phragmidium* (Jour. Jap. Bot. **7**: 227–299. 1935).

Observations of Cheo's collections referable to the section *Phragmotelium* reveal a character which appears not to have been recognized previously. There is a septum present which separates the pedicel proper from a basal portion which is of approximately the same diameter but thinner-walled. Normally the basal portion does not remain attached to the pedicel but can be seen readily in carefully prepared mounts (FIG. 9). An examination of other available species indicates that the septate pedicel may be characteristic of all species of the section.

PHRAGMIDIUM RUBI-THUNBERGII Kusano (FIG. 9). On *Rubus thunbergii* Sieb. & Zucc.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1261*). On *Rubus* sp.: KWEICHOW: Tsunyi Hsien, July, Aug. 1931, (*23, 32, 206*).

*PHRAGMIDIUM NAMBUANUM Diet. On *Rubus* sp.: KWEICHOW: Chiang K'ou Hsien, Oct. 1931, (*658*).

PHRAGMIDIUM MUCRONATUM (Fr.) Schl. On *Rosa* sp.: KWEICHOW: Chiang K'ou Hsien, Oct. 1931, (*739*).

PHRAGMIDIUM ROSAE-DAHURICAE Miura. On *Rosa* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1381, 1392*).

No material of this species has been available for comparison but Cheo's specimens agree well with Miura's description. The teliospores (FIG. 10) are chestnut-brown but not opaque and the telia, likewise, are not blackish brown as in most species on roses.

LEUCOTELIUM PRUNI-PERSICAE (Hori) Tranz. On *Prunus persica* (L.) Stokes: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (*1112*); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*463*). On *Prunus* sp.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (*371*).

As pointed out by Butler and Bisby (Fungi of India, p. 72. 1931), Tai (Nanking Jour. 2: 172. 1932), and Tranzschel (Conspectus Ured. URSS, p. 238. 1939) the urediospores described for *Puccinia pruni-persicae* by Hori (Phytopath. 2: 144. 1912) are actually those of *Tranzschelia pruni-spinosae* and have no connection with the telia. The uredia of the species are pale brownish and have capitate paraphyses somewhat as in *Tranzschelia,* but the urediospores are pale brownish or yellowish, the wall is uniformly echinulate, and uniformly 1.5 μ in thickness.

*MARAVALIA ACHROA (Syd.) Arth. & Cumm. On *Dalbergia* sp.: KWEICHOW: Sze Nan Hsien, Aug. 1931, (*327*).

THE ARTHUR HERBARIUM,
PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION,
LAFAYETTE, INDIANA

DESCRIPTION OF FIGURES

FIG. 1, telium of *Melampsora aleuritidis* Cumm. on *Aleuritis* sp. (from type); 2, telium of *Phakopsora fici-erecti* Ito & Otani on *Ficus* sp. (from Cheo 961); 3, telium of *Phakopsora malloti* Cumm. on *Mallotus* sp. (from type); 4, telium of *Phakopsora cheoana* Cumm. on *Cedrela sinensis* (from type); 5, telium of *Melampsora larici-epitea* Kleb.? on *Salix* sp. (from Cheo 1518). All photos are from unstained free-hand sections. × 800.

FIG. 6, unstained free-hand section of a telium of *Pucciniostele hashiokai* (Hirats. f.) Cumm. on *Ampelopsis* sp. (from Cheo 2939), 7, a single teliospore of *Pucciniostele hashiokai* showing the outer wall swollen over the germ pores; 8, teliospore of *Phragmidium griseum* Diet. on *Rubus* sp. (from Cheo 1123); 9, teliospore of *Phragmidium rubi-thunbergii* Kusano on *Rubus* sp. (from Cheo 23); 10, teliospores of *Phragmidium rosae-dahuricae* Miura on *Rosa* sp. (from Cheo 1381). × 800.

NOTES AND BRIEF ARTICLES

MUSHROOMS IN THEIR NATURAL HABITATS, by Alexander H. Smith. Two volumes: Vol. I, Text (I–XIV + 626 pp.) with eleven text-figures (line-drawings); Vol. II, Illustrations (Stereo-photographs by William B. Gruber). Published in 1949 by Sawyers, Inc., Portland, Oregon (Price $26.50).

This is a new and a successful approach to the study of mushrooms and their relatives. The work is distinguished by the fact that it combines natural-habitat color stereo-photographs with technical descriptions. It is clearly an attempt to improve on the art of presenting to the public authentic and interesting information on the agarics and other fleshy forms in both the ascomycetes and basidiomycetes. The attainment of this goal is facilitated by the author's readable style. The volumes are not designed primarily for the specialist but rather for those of less experience. But the book promises to assist in the identification of certain fleshy fungi, to stimulate an interest in them, and to help one gain a basic understanding of this engaging group of organisms.

Volume I is text; volume II is really a plush-lined box containing a Sawyer's view-master and an album of 33 reels of color photographs, made in the field and woods. As for the text (Volume I), about two-thirds of its pages are devoted to the salient facts relative to each species included, embracing synonomy and a technical description, and a discussion of edibility, habit, habitat, and distribution. Several keys to groups, genera, and species are included. The section on agarics constitutes more than half the text.

There are also many pages of fresh discussion bearing on the subjects of collecting, techniques for microscopic study, nomenclature, and mycophagy; some five pages of literature; a glossary of more than six hundred items; and an adequate index.

Mycologists, especially agaricologists, will welcome the chapter entitled: "Laboratory Techniques and the Study of Microscopic

Characters of Importance in the Classification of Fleshy Fungi." In that section of some forty-five pages, Dr. Smith describes the use of chemical reagents, the microscopic characters of agarics including his morphological grouping of spores, the technique of fruit-body study, and a good discussion of the "tissues,"—hymenium, gill trama, pileus, and stipe. The principal microscopic structures are illustrated by line-drawings in eleven text-figures.

As the author has proceeded in his agaric studies over the last twenty years, he has met with hundreds of species and has felt the inadequacy of the prevailing methods and techniques for distinguishing species. Mere morphological descriptions of the gross features along with spore-dimensions have proved to him and us insufficient to separate certain species which are obviously distinct. He has, in recent years, been willing, if not constrained, to extend inquiry further into the details of microscopic structure of the pileus, stipe, hymenium, and gill trama in an effort to bring all usable characters into play for taxonomic purposes. He has also, in recent years, investigated and extended the use of chemical reagents in an attempt to discover the clincher in a taxonomic problem. His experience in these matters shows up throughout the text, and is clearly suggestive of new approaches to a more satisfactory agaric taxonomy.

Thus, one is impressed with the new features of the two volumes: the subject is treated in accordance with modern research methods and concepts; and the illustrations are stereo-photographs in natural color (mostly excellent!) and habitat. Moreover, the descriptions in the text were drawn chiefly from the particular specimens illustrated. The collections, of course, have been preserved, and are on deposit in the University of Michigan Herbarium.

The author treats 230 species, the selection of which was determined by a number of considerations: those species common to the Northern United States and Southern California (the war curtailed efforts to get photographs in the Southeast and in the Gulf States); special effort was made to include endemics; representatives of as many genera as possible are included; both edible and poisonous species are represented; and finally, no species is treated unless a suitable natural-habitat photograph could be secured.

Wherever justified, in the opinion of the author, he breaks away from conventional classification and nomenclature. Thus, he uses XEROMPHALINA, based on *Omphalia campanella* Quélet; OMPHALINA, including only the section Collybiariae of *Omphalia;* CATATHELASMA, including species of *Armillaria* exhibiting bilateral gill trama and amyloid spores; CYSTODERMA, embracing those species of *Lepiota* (and *Armillaria*) with a granulose covering of the pileus and lower portion of the stipe; LEUCOPAXILLUS, comprising certain species of *Clitocybe, Tricholoma,* and *Pleurotus* with amyloid spores; LYOPHYLLUM, based on certain species of *Collybia, Mycena, Clitocybe,* and *Tricholoma* which exhibit small bodies in the basidia or base of the sterigmata which stain very dark in ferric aceto-carmine; CONOCYBE, species of *Galera* with a cellular to hymeniform cuticle of the pileus; ROZITES, a *Cortinarius* with a rudimentary volva and a membranous partial veil; GYMNOPILUS, "a logical aggregation of species formerly grouped in *Pholiota, Flammula,* and *Naucoria*"; NAEMATOLOMA, fleshy species of *Hypholoma;* PSEUDOCOPRINUS, *Coprinus*-like agarics, the gills of which are non-deliquescent; and also LACCARIA, VAGINATA, LIMACELLA, RHODOPHYLLUS, and GALERINA. The genus CANTHARELLUS is placed in the family Cantharellaceae, along with *Craterellus, Leptotus,* and *Arrhenia.*

The author reports one new species, **Cystoderma Gruberianum,** on page 363 of the text; and one new variety, **Lepiota Molybdites** (G. Meyer ex Fr.) Sacc. var. **marginata,** on page 429. Latin descriptions are provided—to the joy of several, and perhaps to the displeasure of the non-conformists.

The International Rules of Botanical Nomenclature (1930) are followed, in order, as Smith says, to give validity and stability to naming.

Those who have followed the fortunes of the author of this book will be pleased with this his most recent effort. It is a novel two-volume publication, and precisely hits the intended mark. The work is indeed excellent, one which will prove unusually valuable for all who would know the mushrooms and other fungi.—L. R. HESLER.

DIE PILZE, Grundzüge ihrer Entwicklungsgeschichte und Morphologie by Dr. Ernst Gaümann. Lehrbucher und Monographien aus dem Gebiete der Exakten Wissenschaften n. 19. E. Birkhauser & Cie. Elisabethen Strasse 15, Basel, Switzerland. Pp. 1–382. 440 figs. 1949.

All mycologists will welcome this revised treatment on the developmental history and morphology of the fungi by the noted Swiss mycologist. The text, in German, is distinguished by its clarity and preciseness of statement. A remarkable feature is that the author has treated his subject in 382 pages replete with illustrations. The brevity of the treatment of many groups will probably be deplored by some specialists, but one seeking an over all treatment of the fungi will appreciate the evenness of the treatment. The devotion of a little over a page to the whole group of Fungi Imperfecti is in keeping with the author's thesis that when the fungi have all been arranged into a natural system there should be nothing left in the Imperfecti.

In Gaümann's scheme of the evolution of the fungi, see p. 10, the Siphonales and Flagellatae are the two points of origin of the fungi. The line from the Siphonales leads to the Oomycetes where it ends.

The Archimycetes, derived from the Flagellatae, are characterized by lacking a cell wall, living parasitically in the cells of the host, and by the thallus being holocarpic. They are considered to be an endpoint of development at a level below that of the Phycomycetes.

The main line of development leads through the Chytridiales from the Flagellatae. At the Chytridiales it divides into three branches, all of equal level. These are the Monoblepharidales, Blastocladiales, and Zygomycetes. These in addition to the Oomycetes, constitute the group known as the Phycomycetes. All are characterized by a cell wall.

The Ascomycetes are derived from the Phycomycetes through the Zygomycetes. The two most primitive groups are the Taphrinales and Endomycetales. The Taphrinales represent an endpoint of development whereas the Plectascales are derived through the Endomycetales and represent a higher level of development. From the Plectascales numerous branches lead to the various well known orders of Ascomycetes, including the Clavicipitales. The Tuberales are derived from the Pezizales, and constitute the most highly developed group of Ascomycetes.

The Basidiomycetes are derived from the Ascomycetes from the vicinity of the Pezizales and Helotiales. The Gasteromycetes are derived from the Corticiaceae in the Hymenomycetes. The Auriculariales, Tremellales, Hymenomycetes and Gastromycetes are represented as of approximately comparable levels of development with the Ustilaginales and Uredinales as the most advanced Basidiomycetes, and derived from the Auriculariales.

Whether one agrees with this scheme in its entirety or not, it must be admitted that the author has handled his material well and produced a well balanced treatise which will always be an excellent point of departure for a detailed discussion of individual orders and families.—ALEXANDER H. SMITH.

A note from Dr. Bisby states that the third edition of "A Dictionary of the Fungi" has been issued. The price is three dollars ($3.00). It can be obtained from the Imperial Mycological Institute, Kew, Surrey, England.—A. H. SMITH.

A correction: In the article on "Pullularia," p. 436, in lines 8–10, the letters A and B were reversed. A is the panel showing mold growth whereas B is clear of growth.—ERNEST S. REYNOLDS.

MYCOLOGIA

FINANCIAL STATEMENT

(July 1, 1949–June 30, 1950)

Current receipts (joint funds):		
Mycological Society (member's subscriptions)	$2,048.00	
Subscriptions	3,742.82	
Sale of back sets (vol. 25 and later)	382.63	
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Special funds:		
Sale of back sets (vol. 1–24)	$ 176.50	
Interest on endowment	635.00	
	$ 811.50	
Total income		$ 7,868.05
Cost of printing and distribution:		
Printing, binding, mailing	$6,315.94	
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	$7,795.11	
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Adjustment—July 1, 1950	$ 11.22	
Office expense	162.44	
Total expense		$ 7,968.77
Excess of expense over income		(100.72)
Unexpended reserve July 1, 1949	$4,716.23	
Adjustment—July 1, 1949	33.97	
		$ 4,750.20
Unexpended reserve, June 30, 1950		$ 4,649.48
Endowment fund		14,000.00
Total		$18,649.48

The above MYCOLOGIA funds are administered by the New York Botanical Garden, and the balances at June 30, 1950, are in agreement with the amounts shown in the financial statements of that organization which have been examined by Price, Waterhouse & Co.

DONALD P. ROGERS,
Managing Editor

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* This index prepared by Henry A. Imshaug.

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